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EFFECT OF DIETARY TRACE ELEMENT LEVEL AND HOT ENVIRONMENTAL TEMPERATURE  
ON TRACE ELEMENT NUTRITION OF HOLSTEIN DAIRY CATTLE

SHINICHI KUME

1987

Effect of Dietary Trace Element Level and Hot Environmental Temperature  
on Trace Element Nutrition of Holstein Dairy Cattle

Shinichi KUME

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## Chapter 1. Introduction

It is difficult to find a meaningful classification for the trace elements and the major elements, which is based on the amounts required in the diet and in the animal body. At the present time, 27 of 90 naturally occurring elements are known to be essential or probably essential for animal ( 230 ). These consist of 11 major elements, namely, carbon ( C ), hydrogen ( H ), oxygen ( O ), nitrogen ( N ), sulfur ( S ), calcium ( Ca ), phosphorus ( P ), potassium ( K ), sodium ( Na ), chlorine ( Cl ), and magnesium ( Mg ), and 16 trace elements, namely, iron ( Fe ), zinc ( Zn ), copper ( Cu ), manganese ( Mn ), nickel ( Ni ), cobalt ( Co ), molybdenum ( Mo ), selenium ( Se ), chromium ( Cr ), iodine ( I ), fluorine ( F ), tin ( Sn ), silicon ( Si ), vanadium ( V ), arsenic ( As ), and cadmium ( Cd ). In the future, other elements may be recognized as essential for animals, since the study on trace element nutrition has been improved rapidly.

Interest in trace elements in animal physiology began over a century ago with the discovery in living organisms of special compounds involving Fe, Zn, Cu, I, and V ( 230 ). During 1930's a wide range of nutritional disorders of farm stock were found to be caused by deficient or excessive intakes of various trace elements, such as F, Cu, Se, Co, Mn, and Mo, from the natural environment ( 230 ). In more recent years, one of the major reasons for increased research in trace element nutrition have been dependent on the developments in analytical technics, primally such as atomic absorption spectrophotometry, neutron activation, microelectron prove procedures, etc.( 230 ). Recently, there has been a rapid increase in the number of trace elements, such as Sn, Si, F, Ni, V, As, and Cd, shown to be essential or probably essential in animal nutrition ( 213 ).

In Japan, UESAKA et al. ( 43, 218 ) reported in 1953 that there was on

old local disease of Co deficiency in the cattle which has been called " Kuwazu disease " in Shiga prefecture and the other areas. At the same time and thereafter, Cu deficiency or Mo toxicity in the cattle had been found to exist in many areas in Japan ( 13, 52, 55-57, 163, 226 ), and trace element content in milk and roughage had been reported ( 53, 54, 71-77 ). Also, since the discovery of Co deficiency by UESAKA et al., a series of the study on trace element nutrition in ruminants has been reported by UESAKA, KAWASHIMA, and his school ( 49, 78-81, 91-93, 200, 218-228, 239, 240 ).

Recently, most of the book and review on trace element nutrition in ruminants has been published ( 3, 8, 9, 11, 44, 46, 48, 61-64, 92-94, 123, 137, 141-143, 149, 150, 160, 188, 213, 229-231, 233, 236 ). According to those review, trace elements act primally as a critical role of components in the body and catalysts in enzyme systems in the cells. Several elements have very specific roles, where essential functions of Fe and Co are as components of hemoglobin and vitamin B<sub>12</sub>, respectively. Also, enzyme systems of trace elements are generally either metalloenzyme or metal enzyme complexes.

The minimum requirement and the maximum tolerances of animals for the essential trace elements are arrived at by relating the growth, health, fertility, or other relevant criteria in the animal to varying dietary mineral concentrations ( 3, 142, 149, 230 ). Both requirement and tolerance for minerals are influenced by many factors including age and function of the animal, level of performance, body weight or composition of gain, chemical form in feed, levels of other dietary nutrients and additives, various environmental conditions, etc. ( 230 ). However, the requirement and tolerance of trace elements for dairy cattle has not been determined exactly because of a little information in trace element nutrition on ruminants ( 3, 142, 149, 230 ).

Plant materials provide the main source of minerals, and only small amounts are obtained from water, soil, and nonfeed contamination ( 3, 142, 149, 230 ). Also, trace element content of feeds is extremely variable compared to total digestible nutrient or protein, and differences are wide between low and high values for many trace elements within a given type of feed ( 2 ). However, the remarkable adaptability of ruminants is possible to a great variety of feeds, because there is good homeostatic control mechanisms for mineral nutrition of ruminants ( 230 ). Thus, trace element concentration in animal tissues may be maintained within narrow limits because of the control of the absorption, transport, excretion, and tissue turnover of trace elements, if the growth, health, and the productivity of the animal are to be sustained ( 142, 230 ).

However, trace element deficiency or toxicity are most frequently observed in grazing ruminants throughout the world ( 3, 142, 149, 230 ). Furthermore, a marginal deficiency or a toxicity of an essential mineral is highly to reduce milk production, growth rate, resistance to disease or infection, and reproduction by a small percentage ( 142, 230 ). Thus, borderline problems are more costly overall in practical feeding of dairy cattle, because they are so much more widespread ( 142 ).

In recent years, it has been found that Zn, Cu, Se, and Co concentrations in most of Japanese pasture and forage were below the dietary requirement for dairy cattle according to the investigation of trace element concentration in Japanese pasture and forage ( 14,15, 99-104, 152, 162, 200, 203-207 ). Also, it has been studied to clarify the effect of dietary trace element level on nutritional status in ruminants ( 4, 80-82, 106, 107, 109, 146, 164-166, 208, 214-216 ). However, it has not been cleared in Japan to clarify the effect of dietary trace element level on trace element nutrition of dairy cattle, although trace element requirements and tolerances of dairy cattle were published in NRC and ARC



standards ( 3, 149 ). Therefore, it may be necessary to clarify the trace element nutrition of dairy cattle in Japan.

Also, environment directly and indirectly influences the productivity of dairy cattle, and heat stress in the summer environment in Japan causes significant declines in feed intake, milk yield, and milk composition of dairy cattle ( 193, 194 ). Since dairy cattle increase their respiration rate and rectal temperature when exposed to high environmental temperature ( 16, 27, 193 ), it is important to prevent body temperature rise at high temperatures in order to maintain the same productivity as at optimal temperatures.

Heat-stressed dairy cows may have increased K and Na requirements, since the increase in skin K loss and urinary Na loss in hot weather may be important to total economy ( 3, 27 ). Also, since it is suggested that increasing dietary K and Na for heat-stressed cattle may affect milk production and composition, and the physiological and endocrine responses of cattle ( 27, 30, 31, 39, 115, 190, 191 ), it may be possible to increase the productivity of heat-stressed cattle when there is increased dietary K and Na. However, trace element requirements of cows in hot weather have not been well determined, although those in temperate conditions have been estimated for different classes ( 3, 149 ). Therefore, an evaluation of trace element requirements in hot weather is necessary to increase the productivity of dairy cows in Japan.

With the rise of modern technology, the sources of trace elements may affect long-term dangers to human health, since trace element toxicity for human, such as Minamata disease and Itai-itai disease, and the contamination of trace element in food was occurred in Japan ( 151 ). Thus, more attention must be paid to trace element contamination in food and environment in Japan. Furthermore, it may be important to clarify the soil-plant-animal interrelations of trace element because of preventing the

trace element contamination in food, although it has been reported that trace element concentrations in milk and meat was lower than those in the other food ( 69, 70, 83, 108, 147, 148, 209-212 ).

The object of this study was, therefore, to clarify the effect of dietary trace element level and environmental temperature on trace element nutrition of Holstein cattle in order to increase the productivity of dairy cattle in Japan. Firstly, this study was conducted to clarify the effect of dietary level of 8 trace elements, such as Fe, Zn, Cu, Mn, Se, Mo, Co, and Cd, on those status of 37 Holstein cattle by the determination of those concentration in feed and tissues of cattle obtained from Kyushu Natl. Agric. Exp. Stn. and 10 dairy farms in Kumamoto prefecture. Secondly, the ingestion, accumulation, distribution, and excretion of 7 trace elements, such as Zn, Cu, Mn, Se, Mo, Co, and Cd, in Holstein cattle was investigated by the administration of each element for lactating cows. At last, dry and lactating cows were exposed to hot weather in order to clarify the effect of hot environmental temperature on trace element metabolism of cows.

## Chapter 2. Effect of dietary trace element level on trace element nutrition of Holstein cattle

### Section 1. Effect of dietary Fe level on Fe nutrition of cattle

A major function of Fe is as part of the hemoglobin, an Fe-protein complex which carries oxygen from the lungs to tissues and carbon dioxide on the return trip ( 142, 230 ). Although a major portion of the total body Fe is in hemoglobin, Fe also plays a key role in other enzymes involved in oxygen transport and the oxydative processes including catalase, peroxidase, flavoprotein enzymes, and cytochromes ( 142, 230 ). The main problem involving Fe in the practical feeding of dairy cattle is the deficiency which occurs when calves are fed only milk for a substantial period ( 17, 20, 21, 149 ). Also, Fe deficiency seldom occurs in older cattle unless there is considerable blood loss from nonnutritional cause such as parasitic infections or disease ( 149, 152, 204 ).

Although Fe content in Japanese pasture is almost above the 50 ppm dietary Fe requirement for dairy cattle ( NRC ( 149 ) ), it may be necessary to evaluate Fe nutrition of cattle because of mineral interaction among Fe and other elements ( 142, 230 ). The objective of this section was, therefore, to clarify the effect of dietary Fe level on the Fe status of Holstein cattle .

### Materials and Methods

#### 1. Investigation of Fe concentration in feed and tissues of cattle.

Two male Holstein newborn calves and 17 female Holstein cattle kept at Kyushu Natl. Agric. Exp. Stn., ranging in age from newborn to 12 years and from 36 to 741 kg in body weight, were slaughtered from March 1979 to October 1980. Liver and kidney of 19 cattle were taken, while other

tissues of 5 of 19 cattle were also taken immediately after slaughter. In 14 cows from 17 cattle, blood was obtained immediately after slaughter and feces was collected for 1 week before slaughter. Feed samples were taken randomly.

Fourteen cows from 17 cattle were classified into 3 groups for different physiological conditions: dry, lactating, and fattening. They were fed on dry lot and feed intake is shown in Table 1. Also, they were received water and mineral salt as a block ad libitum, and mineral salt contained the following trace elements ( in g per 100 kg ):  $\text{Fe}_2\text{O}_3$ , 143;  $\text{ZnO}$ , 12;  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 39;  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 36;  $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ , 48;  $\text{KI}$ , 20;  $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ , 24. The dry cows ranged in dry period from 2 to 31 months. The lactating cows ranged from 1 to 4 months after parturition, and daily milk yield at the slaughter were 8–17 kg. The fattening cows had been fattening for 5 months.

Eighteen Holstein cows fed in 10 dairy farms in Kumamoto prefecture, ranging in age from 2 to 11 years and in body weight from 380 to 775 kg, were slaughtered from October to November 1979. Liver and kidney were taken immediately after slaughter, and feces and feed samples were collected the day before slaughter.

## 2. Fe analysis of samples

Samples of tissues were freeze-dried and ground by stainless-steel grinder. Samples of blood were freeze-dried and ground by mortar. Samples of feces and feed were dried by  $60^\circ\text{C}$  forced air oven and ground by Wiley mill.

Samples were digested in nitric-perchloric acid and Fe concentration was determined by atomic absorption spectrophotometry ( 187 ). Each value is expressed on a dry matter basis. Also, statistical differences were evaluated by Students  $t$ 'test.

## Results

Iron concentration in feed at the station is shown in Table 2. Iron concentration in beet pulp and Italian ryegrass was higher than that in concentrate and Tall oatgrass, but that in Italian ryegrass varied widely. Iron concentration in water was below 0.1 ppm. From the figures for Fe concentration in feed and feed intake, it was assumed that Fe levels in the total diets of dry, lactating, and fattening cows were 450–1500, 350–1200, 150–175 ppm, respectively.

Iron concentration in the liver, kidney, blood, and feces of cows at the station is shown in Table 3. There were no significant differences between groups in Fe concentration in the liver and feces, but that in the liver of dry cows was higher than for lactating and fattening cows. Also, Fe concentration in the kidney of dry cows was significantly higher than for fattening cows, and that in the blood of dry cows was higher than for lactating cows.

Iron concentration in the liver and kidney of 19 cattle kept at the station is shown in Fig.1. Iron concentrations in the liver and kidney were 29–395 and 29–549 ppm, respectively in 2 newborn and one 1 month old calves, and those in 2 heifers were 87–397 and 256–315 ppm, respectively. Also, those in 14 cows were 138–4123 and 111–1180 ppm, respectively. Iron concentration in liver and kidney of cows varied widely and that was slightly higher than for calves, but not affected by age.

Iron concentration in samples of cows and feed obtained from 10 dairy farms in Kumamoto prefecture is shown in Table 4. Iron concentration in roughage varied enormously and that was higher than that in concentrate. Iron concentration in the liver, kidney, and feces on dairy farms was almost similar to that of lactating and fattening cows at the station.

Iron concentration in the tissues of 5 cattle at the station is shown

in Table 5. Iron concentration in the tissues of a newborn calf was lower than for cows. Also, Fe concentration in the spleen was highest, and that in the liver, kidney, and lung was higher than that in other tissues.

## Discussion

Iron level of 100 ppm in the dry diet should be adequate for all needs of calves to 3 months of age with 50 ppm sufficient for other dairy cattle ( 7, 17, 118, 149, 159 ). Since Fe concentration in feed was above 50 ppm, Fe intake by the cattle in this study was adequate. Iron deficiency seldom occurs in older dairy cattle unless severe loss of blood caused by parasitic infections or disease ( 149 ). Furthermore, Fe intake by the cattle may be sufficient in practical feeding of dairy cattle because of soil ingestion or soil contamination in hay and silage, since Fe concentration in soil was very high. Therefore, except for calves, Fe deficiency in dairy cattle seldom occurs in Japan.

Iron toxicity is not a common problem in feeding dairy cattle, but a sufficient high Fe level in feed or water is determined ( 25, 33, 106, 142, 230 ). It is believed that cattle generally can tolerate 1000 ppm dietary Fe under most conditions, especially if Fe is from natural feed sources and adequate levels of other minerals are supplied ( NRC ( 149 ) ). Iron concentration in roughage, especially in silage, was sometimes above 1000 ppm in this study and other report ( 104 ) because of soil contamination. When excess of an element is fed and absorbed, some may be deposited either as a readily usable reserve and/or in a harmless, but not readily mobilizable, form ( 230 ). There was appreciable storage of Fe in the spleen, lung, liver, and kidney in this study, and Fe concentration in the kidney and blood was affected by dietary Fe level. Furthermore, most of dietary Fe seems to be excreted in the feces, since Fe concentration in

the feces was higher than dietary Fe level. Accordingly, it seems likely that the changes in Fe absorption and the substantial amounts of body stores are tremendously beneficial in permitting cattle to avoid a toxicity. However, it may be necessary to evaluate the effect of high Fe concentration in feed on the utilization of other minerals in cattle because of mineral interaction in the body ( 65, 88, 142, 230 ).

The main problem involving Fe nutrition in the practical feeding of dairy cattle is the deficiency which occurs when calves are fed only milk for a substantial period ( 17, 20, 21, 149 ). In this study, Fe concentration in the liver and kidney of a newborn calf born from cows fed a high Fe diet was lower than for cows. Therefore, it may be needed in providing optimum Fe nutrition for calves in Japan.

Table 1. Feed intake of Holstein cows kept at Kyushu Natl. Agric. Exp. Stn.

Feed	Feed intake ( kg/day )		
	Dry <sup>1)</sup>	Lactating <sup>1)</sup>	Fattening
Concentrate <sup>2)</sup>	1	4-6 <sup>3)</sup>	10-14
Beet pulp	-----	1-2	-----
Italian ryegrass ( hay )	ad libitum	ad libitum	-----
Italian ryegrass ( low moisture silage )	ad libitum	ad libitum	-----
Tall oatgrass ( hay )	-----	-----	2.3-5.5

1) Dry and lactating cows were fed 7-10 kg Italian ryegrass on a dry matter basis. 2) It contained ( in kg per 100 kg ): barley, 40.0; linseed meal, 20.0; corn, 11.0; rice bran, 10.0; wheat bran, 10.0; soybean meal, 5.0; calcium carbonate, 2.0; NaCl, 1.5; dicalcium phosphate, 0.45; and ( in g per 100 kg ): FeSO<sub>4</sub>, 7.50; MnSO<sub>4</sub>, 7.25; CuSO<sub>4</sub>, 0.75; ZnSO<sub>4</sub>, 0.25; CoCl<sub>2</sub>, 0.05; KI, 0.038. 3) Range of feed intake.



Table 2. Iron concentration in feed obtained from  
Kyushu Natl. Agric. Exp. Stn.

Feed	Number of samples	Fe concentration <sup>1)</sup>
Concentrate	4	190 $\pm$ 75 <sup>2)</sup>
Beet pulp	2	487 $\pm$ 13
Italian ryegrass ( hay )	2	474 $\pm$ 402
Italian ryegrass ( low moisture silage )	2	1618 $\pm$ 938
Tall oatgrass ( hay )	2	70 $\pm$ 1

1) ppm on dry matter basis. 2) Mean $\pm$ S.D.

Table 3. Iron concentration in liver, kidney, blood and feces of  
Holstein cows kept at Kyushu Natl. Agric. Exp. Stn.

	Dry	Lactating	Fattening
Number of cows	6	4	4
Dietary Fe level <sup>1)</sup>	450-1500 <sup>2)</sup>	350-1200	150-175
Liver <sup>1)</sup>	1396±1370 <sup>a)3)</sup>	391±233 <sup>a)</sup>	249±125 <sup>a)</sup>
Kidney <sup>1)</sup>	582±321 <sup>a)</sup>	257±104 <sup>a,b)</sup>	244±40 <sup>b)</sup>
Blood <sup>1)</sup>	2314±106 <sup>a)</sup>	2045±201 <sup>b)</sup>	2339±136 <sup>a,b)</sup>
Feces <sup>1)</sup>	2631±747 <sup>a)</sup>	1714±506 <sup>a)</sup>	2683±868 <sup>a)</sup>

a and b: Values in the same line with different superscripts are  
significantly different (  $P < 0.05$  ) 1) ppm on dry matter basis.

2) Range of dietary Fe level. 3) Mean±S.D.

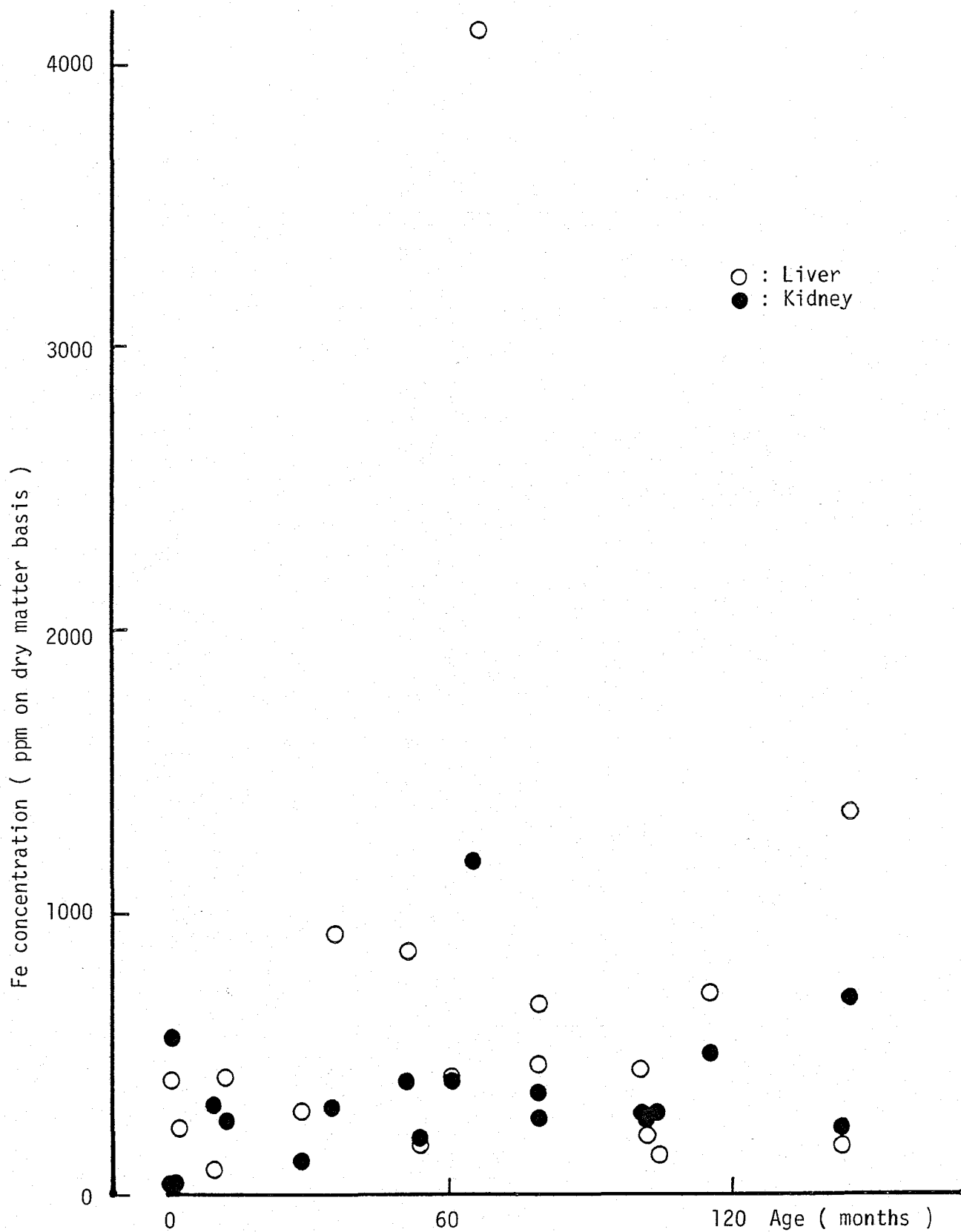


Fig. 1. Iron concentration in liver and kidney of Holstein cattle kept at Kyushu Natl. Agric. Exp. Stn.

Table 4. Iron concentration in samples of Holstein cows and feed obtained from 10 dairy farms in Kumamoto prefecture

Sample	Number of samples	Fe concentration <sup>1)</sup>	
		Mean±S.D.	Range
Cow			
Liver	16	260±156	79-625
Kidney	18	348±135	176-666
Feces	18	2271±1392	859-6183
Feed			
Concentrate <sup>2)</sup>	7	262±214	100-681
Corn	6	1309±1377	130-3960
( soiling and silage )			
Italian ryegrass	1	295	
( hay )			
Mixed grass	1	628	
( hay )			
Wild grass	1	434	
( hay )			
Rice straw	4	240±82	157-317
Citrus pulp	4	164±125	19-317
Tofu pulp	1	141	
Whisky by-product	1	1329	

1) ppm on dry matter basis. 2) Commercial formula feed

Table 5. Iron concentration in tissues of Holstein cattle  
kept at Kyushu Natl. Agric. Exp. Stn

Cattle No. <sup>1)</sup>	1	2	3	4	5
Age ( mos )	newborn	9	65	79	115
Body weight ( kg )	54	205	574	500	690
Liver	29 <sup>2)</sup>	87	4123	462	711
Kidney	36	315	1180	272	493
Heart	94	176	232	186	207
Lung	207	244	2080	1493	2624
Pancreas	---	33	386	337	175
Spleen	390	1101	23899	---	44374
Diaphragm	---	27	23	109	71
M. Biceps femoris	23	13	105	86	110
M. Longissimus thoracis	---	13	31	72	54
Gall bladder	---	7	---	---	44
Rumen	---	82	466	350	322
Reticulum	---	186	---	---	3179
Omasum	---	110	---	---	863
Abomasum	8	255	---	---	342
Small intestine	38	40	80	326	83
Large intestine	8	16	94	10	48
Uterus	---	17	---	---	118
Urinary bladder	---	84	---	---	35
Skin	---	140	---	---	92

1) 5 cattle of different ages from the 19 cattle shown in Fig.1.

2) ppm on dry matter basis.

## Section 2. Effect of dietary Zn level on Zn nutrition of cattle

The average concentration of Zn at about 20 ppm on a fresh basis in the body of dairy animals is much higher than that of any other trace element except for Fe, and Zn deficiency under natural grazing conditions has been reported in several countries ( 3, 123, 142, 149, 230 ). Zinc functions largely or entirely in enzyme systems including both metalloenzymes and metal-enzyme complexes, and Zn plays a key role in carbohydrate metabolism, protein synthesis, nucleic acid metabolism, and in many other biochemical reactions in the body ( 143 ). Although Zn content in Japanese pasture ( 152, 204 ) is almost below the 40 ppm dietary Zn requirement for dairy cattle ( NRC ( 149 ) ), no Zn deficiency in cattle has been reported in Japan. However, feeding low Zn diets of roughage may increase the possibility of Zn deficiency.

The object of this section was, therefore, to clarify the effect of dietary Zn level on the Zn status of Holstein cattle.

### Materials and Methods

#### 1. Investigation of Zn concentration in feed and tissues of cattle

The experiment was conducted as previously described ( Section 1. )

#### 2. Effect of high Zn level in rations on Zn status in the body

Two lactating cows were fed 5 g of Zn as zinc sulfate mixed in their concentrate, daily for 4 weeks. One cow was slaughtered immediately after the administration, while the other one was slaughtered 4 weeks after the termination of the administration. The experiment was performed from 4 weeks before Zn administration to slaughter and consisted of 3 periods: pre-treatment, treatment, and post-treatment. Both cows were fed 4 kg concentrate, 2 kg beet pulp, and Italian ryegrass hay ad libitum. They

received water and mineral salt as a block ad libitum. They were slaughtered at 68 and 44 months old, and 566 and 488 kg in body weight, respectively.

Feces were collected 9 times each period with grab sampling from the rectum, and urine and milk were collected 3 times each period. Blood samples were obtained 2 times each period by jugular puncture. Hair was collected just at the termination of each period from the portion around the scapula. Feed samples were taken randomly. Tissues of treated cows were obtained immediately after slaughter. Five untreated cattle were chosen on the basis of age, and their tissues were obtained as described above.

### 3. Zn analysis of samples

Samples of tissues were freeze-dried and ground by stainless-steel grinder. Samples of milk and blood were freeze-dried and ground by mortar. Samples of feces and feed were dried by 60°C forced air oven and ground by Wiley mill. Hair samples were washed with ethanol and distilled water carefully, and dried by 100°C forced air oven. Urine samples were frozen before analysis.

Samples were digested in nitric-perchloric acid and Zn concentration was determined by atomic absorption spectrophotometry ( 187 ). Each value, except for urine, is expressed on a dry matter basis. Also, statistical differences were evaluated by Students t'test.

## Results

### 1. Investigation of Zn concentration in feed and tissues of cattle

Zinc concentration in feed at the station is shown in Table 6. Zinc concentration in beet pulp, Italian ryegrass, and Tall oatgrass was 2-3 fold lower than that in concentrate. Zinc concentrations in water was 0.2

ppm. From the figures for Zn concentration in feed and feed intake, it was assumed that Zn levels in the total diets of dry, lactating, and fattening cows were 26-32, 31-37, and 38-46 ppm, respectively.

Zinc concentration in the liver, kidney, blood, and feces of cows at the station is shown in Table 7. There were no significant differences between groups in Zn concentration in the liver, kidney, and blood, but that in the liver of lactating and fattening cows was higher than for dry cows. Also, there were significant differences between groups in the feces, and fattening cows had the highest level of Zn in the feces.

Zinc concentration in the liver and kidney of 19 cattle kept at the station is shown in Fig. 2. Zinc concentrations in the liver and kidney were 377-576 and 83-114 ppm respectively in 2 newborn and one 1 month old calves, but those in 2 heifers were 151-356 and 94-118 ppm, respectively. Also, those in 14 cows were 116-341 and 86-131 ppm, respectively. Zinc concentration in the liver of cows was lower than for calves. Also, Zn concentration in the liver was higher than in the kidney, and that in the liver and kidney of cows was not affected by age.

Zinc concentration in samples of cows and feed obtained from 10 dairy farms in Kumamoto prefecture is shown in Table 8. Zinc concentration in roughage was lower than that in concentrate, and that in whisky by-product was very high. Also, Zn concentration in the liver, kidney, and feces on dairy farms was almost similar to at the station.

## 2. Effect of high Zn level in rations on Zn status in the body

Feed intake was not decreased by Zn administration. Daily milk yields of treated cows were 12-15 and 7-11 kg, respectively, and not affected by Zn administration. Zinc concentrations in concentrate, beet pulp, and Italian ryegrass were 51, 22, and 38 ppm, respectively. It was assumed that daily Zn intake and dietary Zn level in the pre-treatment and post-treatment periods were 0.5-0.6 g and 38 ppm, respectively, whereas those of the



treatment period were 5.5 g and 400 ppm, respectively.

Zinc concentrations in the feces, urine, milk, blood, and hair of 2 Zn-treated cows is shown in Table 9. Zinc concentrations in the feces in the treatment period was about 10 fold higher than before or after treatment. However, Zn concentration in the urine, milk, blood, and hair in the treatment period was almost similar to before or after treatment. Assuming that digestibility of dry matter and daily urine amount were 65 % and 10 kg, respectively, daily Zn excretion in feces, urine, and milk of the pre-treatment and post-treatment periods were 0.4, 0.003, and 0.05 g, respectively, whereas those of the treatment period were 4.5, 0.003, and 0.05 g, respectively.

Zinc concentration in the tissues of treated and untreated cattle is shown in Table 10. Zinc concentration in the liver of a newborn calf was higher than for cows, but in the other tissues of the calf it was similar to that for cows. Zinc was evenly distributed among most tissues except the liver of untreated cattle, and Zn concentration in most tissues of treated cows was almost similar to that for untreated cattle. Also, Zn concentration in the rumen content of the cow slaughtered immediately after the administration was 558 ppm.

## Discussion

The minimum dietary Zn requirement is set as 40 ppm for all classes of dairy cattle ( NRC ( 149 ) ). Since Zn concentration in roughage was a little below 40 ppm, Zn intake by the cattle fed mainly roughage in this study was marginally inadequate. MILLER ( 142 ) has reported that effects such as small reductions in feed intake, growth rate, resistance to infections, and possibly a decreased reproductive efficiency may be expected when Zn is marginally inadequate. Also, the effects of severe Zn

deficiency have been well characterized in young male cattle, but Zn deficiency in mature ruminants has been studied much less ( 122, 124, 125, 128-130, 169 ). No symptoms of Zn deficiency were observed in this study. There may be, however, the possibility of a borderline Zn deficiency for the cattle fed rations of low Zn content as roughage in Japan, because Zn content of roughage was almost below 40 ppm in this study and earlier paper ( 204 ). Furthermore, more attention must be paid to Zn supplementation in feeding dairy cattle, because Zn intake from mineral salt has been shown to be most likely inadequate.

When cattle are fed a low Zn diet, the percentage of the dietary Zn absorbed increase, with values as high as 80 % sometimes absorbed, but with a high Zn diet there is a reduction in the percentage absorbed, sometimes to less than 10 % ( 47, 86, 131, 134, 136, 155, 158, 174, 177, 180, 184, 198, 199 ). Furthermore, endogenous Zn excretion and lactation also contribute to Zn homeostasis in cattle ( 126, 127, 138, 153, 154 ). In this study, Zn concentration in the liver, kidney, blood of the cows was not affected by dietary Zn level. Since Zn concentration in the feces increased with the increase in dietary Zn, most dietary Zn seems to be excreted in the feces. Accordingly, it seems likely that tissue Zn concentration is usually maintained constant, even though Zn intake is marginally inadequate. Thus, Zn deficiency may occur after long periods of feeding low Zn diets.

Feed intake and daily milk yield of treated cows in this study were not affected by the administration of 400 ppm Zn in the diet. The toxicity threshold is estimated to range from 500 to 1500 ppm ( NRC )( 149, 170-173 ). Since Zn concentrations in ration in this experiment and an earlier study ( 204 ) was below 500 ppm, Zn toxicity for cattle may not occur in dairy cattle in Japan, except for industrial Zn contamination of soil and plant.

It has been reported that feeding a high Zn diet results in a great increase in the Zn content in a few tissues including the liver, pancreas, and kidney in calves, but not in cows ( 96, 97, 134, 136, 138, 155, 158, 172, 173, 197, 198 ). This fact coincided with this study, since Zn concentration in the liver of 2 newborn calves born from cows fed a low Zn diet in this study was higher than for cows, but Zn concentration in the tissues of treated cows in this study was almost similar to that of untreated cattle. Also, KINKAID et al. ( 97 ) have reported that failure of tissue Zn to increase sharply in cows fed high dietary Zn may be due to the inability of cow tissues to retain excess Zn and, hence, a greater turnover rate. However, the concentrations of other trace elements in the tissues of cows fed high levels of those were higher than that for untreated cows ( Section 3-8 ). These facts suggest that Zn nutrition of cow is different from other trace elements. Furthermore, the maximum safe levels is probably affected by the amounts of Cu and Fe in the diets, as excessive Zn may aggravate borderline deficiency of those elements ( 142 ). Therefore, further study may be needed on interrelationships that occur between Zn and other trace elements.

Table 6. Zinc concentration in feed obtained from  
Kyushu Natl. Agric. Exp. Stn.

Feed	Number of samples	Zn concentration <sup>1)</sup>
Concentrate	4	51 <u>+</u> 4 <sup>2)</sup>
Beet pulp	2	19 <u>+</u> 4
Italian ryegrass ( hay )	2	29 <u>+</u> 13
Italian ryegrass ( low moisture silage )	2	24 <u>+</u> 3
Tall oatgrass ( hay )	2	14 <u>+</u> 3

1) ppm on dry matter basis. 2) Mean+S.D.

Table 7. Zinc concentration in liver, kidney, blood and feces of Holstein cows kept at Kyushu Natl. Agric. Exp. Stn.

	Dry	Lactating	Fattening
Number of cows	6	4	4
Dietary Zn level <sup>1)</sup>	26-32 <sup>2)</sup>	31-37	38-46
Liver <sup>1)</sup>	160 $\pm$ 30 <sup>a)3)</sup>	242 $\pm$ 96 <sup>a)</sup>	214 $\pm$ 82 <sup>a)</sup>
Kidney <sup>1)</sup>	111 $\pm$ 14 <sup>a)</sup>	112 $\pm$ 11 <sup>a)</sup>	118 $\pm$ 14 <sup>a)</sup>
Blood <sup>1)</sup>	10.4 $\pm$ 2.0 <sup>a)</sup>	10.1 $\pm$ 0.4 <sup>a)</sup>	11.9 $\pm$ 4.4 <sup>a)</sup>
Feces <sup>1)</sup>	65 $\pm$ 11 <sup>a)</sup>	89 $\pm$ 19 <sup>b)</sup>	131 $\pm$ 17 <sup>c)</sup>

a, b, and c: Values in the same line with different superscripts are significantly different (  $P < 0.05$  ) 1) ppm on dry matter basis.

2) Range of dietary Zn level. 3) Mean $\pm$ S.D.

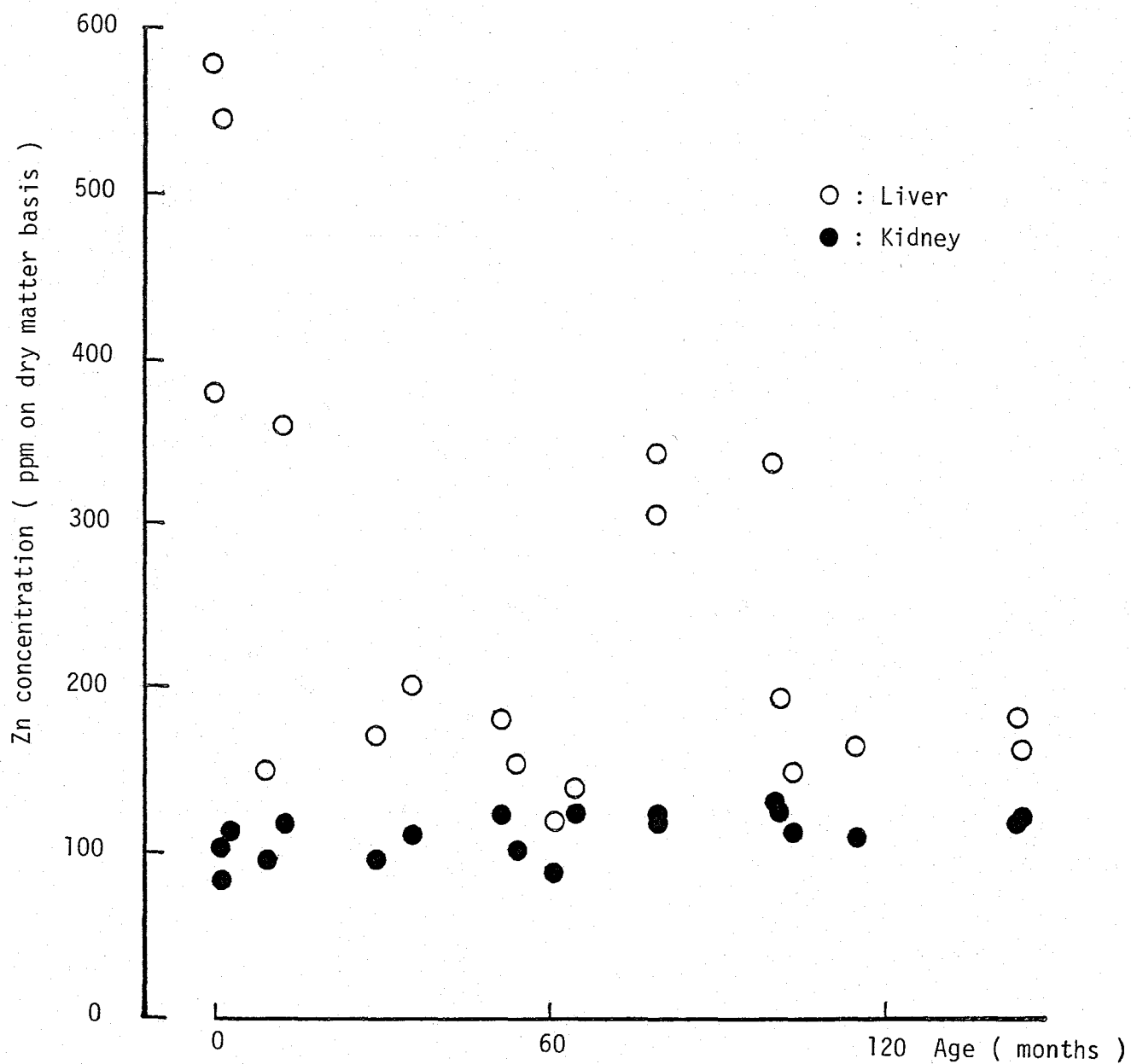


Fig. 2. Zinc concentration in liver and kidney of Holstein cattle kept at Kyushu Natl. Agric. Exp. Stn.

Table 8. Zinc concentration in samples of Holstein cows and feed obtained from 10 dairy farms in Kumamoto prefecture

Sample	Number of samples	Zn concentration <sup>1)</sup>	
		Mean±S.D.	Range
Cow			
Liver	16	156±54	116-299
Kidney	18	104±14	90-135
Feces	18	104±16	76-138
Feed			
Concentrate <sup>2)</sup>	7	60±5	55-68
Corn ( soiling and silage )	6	40±32	18-103
Italian ryegrass ( hay )	1	21	
Mixed grass ( hay )	1	22	
Wild grass ( hay )	1	45	
Rice straw	4	46±4	41-51
Citrus pulp	4	20±12	10-38
Tofu pulp	1	39	
Whisky by-product	1	230	

1) ppm on dry matter basis. 2) Commercial formula feed

Table 9. Zinc concentration in feces, urine, milk, blood, and hair of  
2 zinc-treated cows<sup>1)</sup>

Item	Number of collections <sup>4)</sup>	ZnSO <sub>4</sub> administration		
		Pre-treatment <sup>5)</sup>	Treatment period <sup>5)</sup>	Post-treatment <sup>5)</sup>
Feces <sup>2)</sup>	9	80 $\pm$ 11 <sup>6)</sup>	863 $\pm$ 121 <sup>6)</sup>	81 $\pm$ 8 <sup>7)</sup>
Urine <sup>3)</sup>	3	0.26 $\pm$ 0.11	0.32 $\pm$ 0.12	0.31 $\pm$ 0.01
Milk <sup>2)</sup>	3	25 $\pm$ 7	30 $\pm$ 4	33 $\pm$ 8
Blood <sup>2)</sup>	2	18 $\pm$ 3	19 $\pm$ 2	17 $\pm$ 1
Hair <sup>2)</sup>	1	132 $\pm$ 9	152 $\pm$ 23	142

1) administered 5 g of Zn as ZnSO<sub>4</sub> daily for 4 weeks. 2) ppm on dry matter basis.  
3) ppm on wet matter basis. 4) obtained from a cow. 5) Each period consists of 4  
weeks. 6) Mean $\pm$ S.D. of collections of 2 cows. 7) Mean $\pm$ S.D. of collections of a cow.



Table 10. Zinc concentration in tissues of zinc-treated and untreated cattle

Group	untreated <sup>1)</sup>					treated	
Cattle No.	1	2	3	4	5	7 <sup>2)</sup>	8 <sup>3)</sup>
Age ( mos. )	newborn	9	65	79	115	68	44
Body weight ( kg )	54	205	574	500	690	566	488
Liver	576 <sup>4)</sup>	151	137	305	162	207	159
Kidney	105	94	124	121	108	125	98
Heart	107	46	88	64	68	74	65
Lung	108	79	100	73	109	70	90
Pancreas	---	122	90	137	129	148	144
Spleen	129	120	92	---	62	105	95
Diaphragm	---	126	103	180	124	124	131
M. Biceps femoris	113	114	157	129	166	172	141
M. Longissimus thoracis	---	190	103	147	161	176	152
Rumen	---	132	96	69	119	108	81
Reticulum	---	118	---	---	129	126	129
Omasum	---	134	---	---	158	121	148
Abomasum	86	49	---	---	89	69	63
Small intestine	85	106	84	62	106	88	81
Large intestine	102	101	73	37	94	91	76
Uterus	---	82	---	---	83	70	66
Urinary bladder	---	113	---	---	142	137	95
Skin	---	63	---	---	16	61	46

1) 5 cattle of different ages from the 19 cattle shown in Fig. 2.

2) slaughtered 4 weeks after the termination of the administration.

3) slaughtered immediately after the administration. 4) ppm on dry matter basis.

### Section 3. Effect of dietary Cu level on Cu nutrition of cattle

Initially, Cu was shown to be essential for hemoglobin formation, and subsequent research demonstrated that Cu is crucial in the pigmentation process of hair, bone formation, synthesis of myoglobin, reproduction, normal heart function, formation of connective tissues, and myelination of the spinal cord ( 142, 230 ). Furthermore, Cu is essential in numerous oxidative enzymes including tyrosinase, urinase, ascorbic acid oxidase, cytochrome oxidase, and lysyl oxidase ( 142, 230 ).

Copper deficiency in grazing cattle has become recognized as a major practical problem in many parts of the world ( 3, 123, 142, 149, 230 ), and Cu deficiency or Mo toxicity in ruminants has been reported in Japan ( 13, 52, 55, 163, 226 ). The metabolism, amounts required, and maximum safe dietary level of essential mineral elements are affected in a major way by the level of other minerals in the diets, and Cu deficiency results either from too little Cu per se or from the influence of interfering substances, especially high Mo ( 142, 230 ). Thus, a minimum Cu requirement can not be established without considering the Mo level and, in some instances, other interfering substances, especially sulfate and other materials in pasture forages ( 142, 230 ).

Since Cu content of Japanese pasture ( 152, 205 ) is almost below the 10 ppm dietary requirement for dairy cattle ( NRC ( 149 )), Cu deficiency appears to be the major problem in the practical feeding of dairy cattle. The objective of this section was, therefore, to clarify the effect of dietary Cu level on the Cu status of Holstein cattle.

#### Materials and Methods

##### 1. Investigation of Cu concentration in feed and tissues of cattle

The experiment was conducted as previously described ( Section 1. ).

## 2. Effect of high Cu level in rations on Cu status in the body

Two lactating cows were fed 1 g of Cu as copper sulfate mixed in their concentrate, daily for 4 weeks. One cow was slaughtered immediately after the administration, while the other one was slaughtered 4 weeks after the termination of the administration. The experiment was performed from 4 weeks before Cu administration to slaughter and consisted of 3 periods: pre-treatment, treatment, and post-treatment. Both cows were fed 4 kg concentrate, 2 kg beet pulp, and Italian ryegrass hay ad libitum. They received water and mineral salt as a block ad libitum. They were slaughtered at 90 and 32 months old, and 598 and 461 kg in body weight, respectively.

Feces were collected 9 times each period with grab sampling from the rectum, and urine and milk were collected 3 times each period. Blood samples were obtained 2 times each period by jugular puncture. Hair was collected just at the termination of each period from the portion around the scapula. Feed samples were taken randomly. Tissues of treated cows were obtained immediately after slaughter. Five untreated cattle were chosen on the basis of age, and their tissues were obtained as described above.

## 3. Cu analysis of samples

Preparation of samples was conducted as previously described ( Section 2. ). Samples were digested in nitric-perchloric acid and Cu concentration was determined by atomic absorption spectrophotometry after extraction with methyl isobutyl ketone ( 196 ). Each value, except for urine, is expressed on a dry matter basis. Statistical differences were evaluated by students t'test.

## Results

## 1. Investigation of Cu concentration in feed and tissues of cattle

Copper concentration in feed at the station is shown in Table 11. Copper concentration in Italian ryegrass and Tall oatgrass was 2-6 fold lower than that in concentrate. Copper concentration in water was below 0.01 ppm. From the figures for Cu concentration in feed and feed intake, it was assumed that Cu levels in total diets of dry, lactating, and fattening cows were 4.8-5.8, 6.4-8.0, and 7.8-9.8 ppm, respectively.

Copper concentration in the liver, kidney, blood, and feces is shown in Table 12. Copper concentration in the liver and feces of fattening cows was significantly higher than for dry cows, and that of lactating cows was higher than for dry cows, but not significantly different. Copper concentration in the blood of fattening and lactating cows was significantly higher than for dry cows, and that in the kidney of fattening cows was significantly higher than for lactating cows.

Copper concentration in the liver and kidney of 19 cattle kept at the station is shown in Fig. 3. Copper concentrations in the liver and kidney were 192-231 and 10-17 ppm respectively in 2 newborn and one 1 month old calves, but those in 2 heifers were 39-114 and 22-23 ppm, respectively. Also, those in 14 cows were 4-170 and 14-22 ppm, respectively. Copper concentration in the liver of calves was higher than for cows. Also, Cu concentration in the liver of cows varied widely and that in 5 cows was below 10 ppm. Furthermore, those 5 cows consisted of 4 dry and one lactating cows; and severe diarrhea occurred in the lactating cow. However, Cu concentration in the liver and kidney of cows were not affected by age.

Copper concentration in samples of cows and feed obtained from 10 dairy farms is shown in Table 13. Copper concentration in roughage was lower than that in concentrate; that in whisky by-product was very high. Copper concentration in the liver, kidney, and feces on dairy farms was almost similar to that of fattening and lactating cows at the station.

## 2. Effect of high Cu level in rations on Cu status in the body

Feed intake of one cow was slightly decreased for a few days at the start of Cu administration, but soon after it recovered. However, feed intake of the other cow was not affected by Cu administration. Daily milk yields of treated cows were 11-15 and 9-11 kg, respectively, and not affected by Cu administration. Copper concentration in concentrate, beet pulp, and Italian ryegrass was 14.8, 7.7, and 4.4 ppm, respectively. It was assumed that daily Cu intake and dietary Cu level in the pre-treatment and post-treatment periods were 0.10-0.11g and 7 ppm, respectively, whereas those of the treatment period were 1.1 g and 75 ppm, respectively.

Copper concentration in the feces, urine, milk, and hair of 2 Cu-treated cows is shown in Table 14. Copper concentration in the feces in the treatment period was about 10 fold higher than before or after treatment, and that in the hair in the treatment period was 3-5 fold higher than before or after treatment. Also, Cu concentration in the milk in the treatment period was slightly higher, but that in the urine and blood in the treatment period was almost similar to before or after treatment. Assuming that digestibility of dry matter and daily urine amount were 65 % and 10 kg, respectively, daily Cu excretion in the feces, urine, and milk of the pre-treatment and post-treatment periods were 0.10, 0.0001, and 0.0005 g, respectively, whereas those of treatment period were 1.06, 0.0001, and 0.0008 g, respectively.

Copper concentration in the tissues of treated and untreated cattle is shown in Table 15. Copper concentration in the liver of a newborn calf was higher than for cows, but in the other tissues of the calf it was similar to that for cows. Copper concentration in the liver was highest, and that in the kidney and heart was higher than that in the other tissues. Copper concentration in the liver of treated cows was higher than for untreated cattle, and that of the cow slaughtered 4 weeks after the termination of

the administration was highest. Also, Cu concentration in the gall bladder, rumen, reticulum, omasum, and abomasum of the cow slaughtered immediately after the administration was higher than for untreated cattle, and that in the rumen content was 99 ppm.

## Discussion

According to NRC standards ( 149 ), 10 ppm Cu is a relatively practical minimum requirement for dairy cattle, although 4 ppm Cu will meet requirement under some conditions. Since Cu concentration in roughage was almost below 10 ppm, Cu intake by the cattle fed mainly roughage in this study was marginally inadequate. One of the more important nutritional and biochemical interactions among minerals, especially from the standpoint of grazing animals, is that among Cu, Mo, and S ( 35-37, 116, 117, 144, 168, 202, 230, 232 ). Also, S requirements are related to the amounts of Mo and Cu being ingested or present in the tissues of the animal, and with the grazing animal lack of S is rarely likely to be a significant factor limiting the ability of Mo to reduce Cu retention in the animal ( 230 ). Therefore, attention has focused on the quantitative relations between Cu and Mo and the Cu:Mo ratio ( 230 ). However, from the point of view of Cu and Mo concentration in feed in this study and earlier papers ( 205, 206 ), low Cu concentration in roughage was the most practical problem in feeding dairy cattle, and there may be the possibility of a borderline Cu deficiency for the cattle fed rations of low Cu content such as roughages in Japan.

The criteria most widely used for Cu deficiency are the concentrations in the liver and blood ( 6, 19, 26, 32, 38, 41, 142, 183, 201, 230 ). The liver is the main storage organ of the body for Cu, so that liver Cu concentrations can be expected to provide a useful index of the Cu status

of the animal, and liver Cu concentration below 10 ppm indicates severe deficiency for cows ( 142 ). In this study, Cu concentration in the liver and blood of the cows was affected by dietary Cu level. Furthermore, liver Cu concentration in 5 cows was below 10 ppm, and severe diarrhea occurred in a lactating cow. A wide variety of clinical symptoms have been associated with Cu deficiency, and Cu-responsive diarrhea has been observed in cattle in several parts of the world ( 142, 230 ).

Accordingly, it seems likely that Cu deficiency may occur after long periods of feeding low Cu diets, since Cu concentration in the liver decreased with reduced dietary Cu, and low Cu concentration in the liver was regarded as Cu deficiency in this study.

It is estimated that cattle can safely tolerate 70-100 ppm Cu continuously, and even higher levels for short periods such as a few weeks ( NRC ( 149 ) ). In this study, Cu concentration in feed except for whisky by-product was below 20 ppm, but in whisky by-product it was above 100 ppm. Also, feed intake and daily milk yield of Cu-treated cows in this study were not affected by the administration of 75 ppm in the diet, except for decreased appetite at the start of Cu administration. Since Cu concentration in this study and earlier papers ( 205, 206 ) was almost below 20 ppm, Cu toxicity of cattle may not occur in dairy cattle in Japan except in cases of Cu contamination in soil or plants. However, it is necessary to pay attention to Cu contamination in feed, because Cu is widely used for many agricultural and industrial purposes ( 230 ). Furthermore, most dietary Cu seems to be excreted in the feces, since Cu concentration in the feces increased with an increase in dietary Cu in this study and it is indicated in ARC standards ( 3 ) that a Cu absorption coefficient of 0.06 might be appropriate for cattle given hay and concentrate diets. Copper contamination in feed may, therefore, pose environmental problems, because of the high Cu content of the animal

wastes, from the point of view of the soil-plant-animal interrelations.

When cattle consume excessive Cu, large amounts accumulate in the liver before obvious toxicity symptoms become evident ( 8, 142, 230 ). Also, liver Cu may be maintained for long periods comparatively, since Cu concentration in the liver of Cu-treated cows was very high and that of the cow slaughtered 4 weeks after the termination of the administration was higher than for untreated cattle in this study. Furthermore, Cu concentration in the liver of newborn calves is significantly higher than for dams; liver Cu begins to decrease soon after birth, presumably from mobilization to meet the needs of other tissues ( 63 ). This was indicated in this study, since Cu concentration in the liver of 2 newborn calves born from cows fed low Cu diets in this study was higher than for cows.

The metabolic interaction between Cu and Mo in ruminants was discussed later ( Section 6. ). In addition to Mo and S, other trace elements can influence the Cu requirements of cattle ( 18, 40, 84, 85, 88, 142, 145, 230 ). However, most of the interactions between minerals have not been studied sufficiently with dairy cattle to arrive at quantitative data to define accurately the safe dietary ratio limits. Therefore, further study will provide much needed refinement and tolerance levels of Cu.



Table 11. Copper concentration in feed obtained from  
Kyushu Natl. Agric. Exp. Stn.

Feed	Number of samples	Cu concentration <sup>1)</sup>
Concentrate	4	11.1 $\pm$ 3.2 <sup>2)</sup>
Beet pulp	2	9.2 $\pm$ 0.5
Italian ryegrass ( hay )	2	4.2 $\pm$ 2.1
Italian ryegrass ( low moisture silage )	2	5.1 $\pm$ 0.4
Tall oatgrass ( hay )	2	1.7 $\pm$ 1.1

1) ppm on dry matter basis. 2) Mean $\pm$ S.D.

Table 12. Copper concentration in liver, kidney, blood and feces of Holstein cows kept at Kyushu Natl. Agric. Exp. Stn.

	Dry	Lactating	Fattening
Number of cows	6	4	4
Dietary Cu level <sup>1)</sup>	4.8-5.8 <sup>2)</sup>	6.4-8.0	7.8-9.8
Liver <sup>1)</sup>	20 $\pm$ 27 <sup>a)3)</sup>	76 $\pm$ 68 <sup>a,b)</sup>	112 $\pm$ 16 <sup>b)</sup>
Kidney <sup>1)</sup>	19 $\pm$ 3 <sup>a,b)</sup>	18 $\pm$ 1 <sup>a)</sup>	20 $\pm$ 1 <sup>b)</sup>
Blood <sup>1)</sup>	3.8 $\pm$ 1.1 <sup>a)</sup>	6.3 $\pm$ 1.0 <sup>b)</sup>	5.6 $\pm$ 1.0 <sup>b)</sup>
Feces <sup>1)</sup>	18 $\pm$ 5 <sup>a)</sup>	23 $\pm$ 4 <sup>a,b)</sup>	29 $\pm$ 6 <sup>b)</sup>

a and b: Values in the same line with different superscripts are significantly different ( P 0.05 ) 1) ppm on dry matter basis.

2) Range of dietary Cu level. 3) Mean $\pm$ S.D.

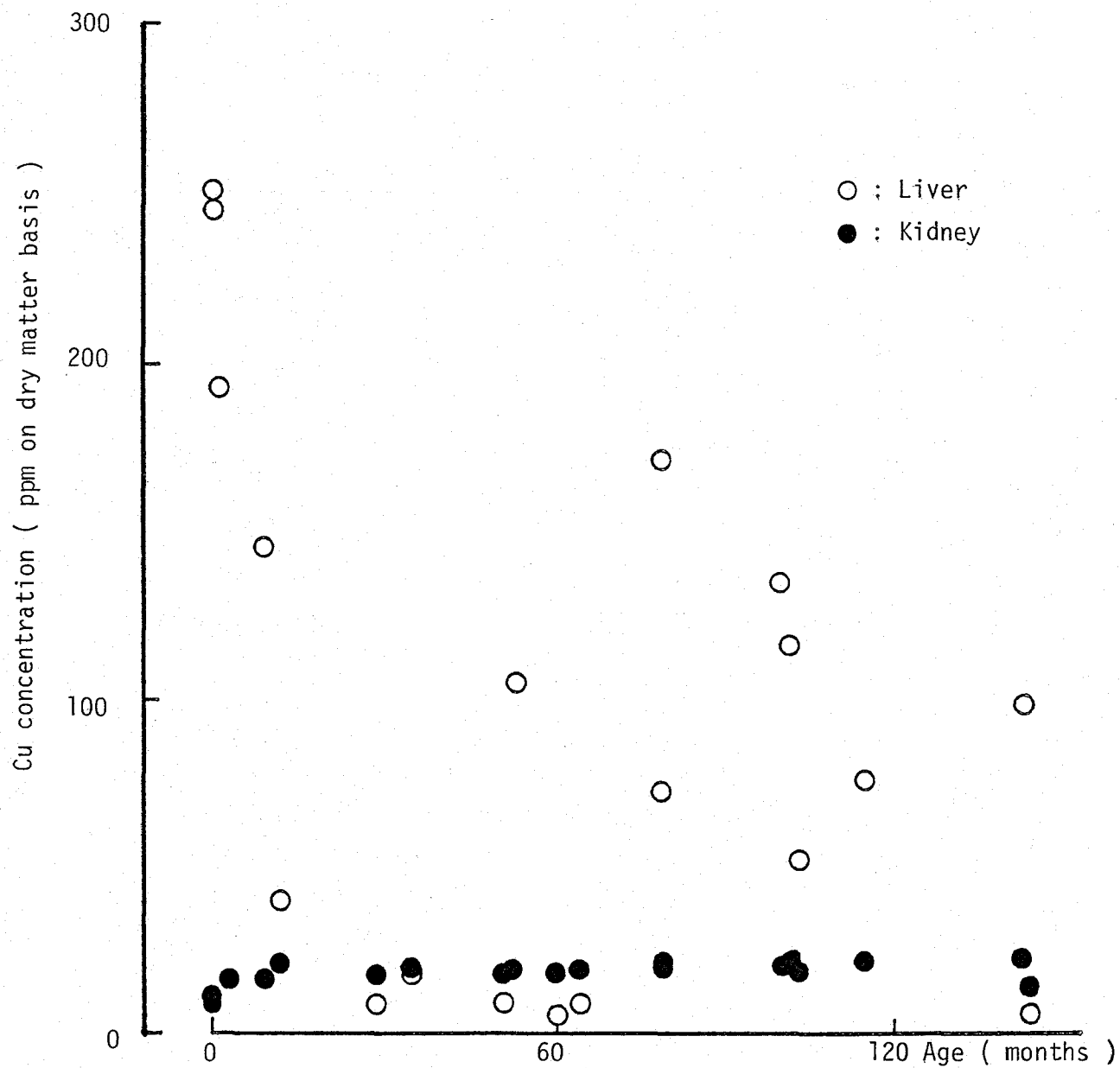


Fig. 3. Copper concentration in liver and kidney of Holstein cattle kept at Kyushu Natl. Agric. Exp. Stn.

Table 13. Copper concentration in samples of Holstein cows and feed obtained from 10 dairy farms in Kumamoto prefecture

Sample	Number of samples	Cu concentration <sup>1)</sup>	
		Mean±S.D.	Range
Cow			
Liver	16	139±97	21-319
Kidney	18	18±2	13-22
Feces	18	23±6	15-34
Feed			
Concentrate <sup>2)</sup>	7	9.5±1.7	7.0-12.4
Corn (soiling and silage)	6	7.7±2.8	3.5-12.1
Italian ryegrass (hay)	1	3.6	
Mixed grass (hay)	1	9.5	
Wild grass (hay)	1	7.0	
Rice straw	4	1.7±0.5	0.9-2.0
Citrus pulp	4	3.0±1.4	1.7-4.7
Tofu pulp	1	13.7	
Whisky by-product	1	114.3	

1) ppm on dry matter basis. 2) Commercial formula feed

Table 14. Copper concentration in feces, urine, milk, blood and hair of 2 copper-treated cows<sup>1)</sup>

Item	Number of collections <sup>4)</sup>	CuSO <sub>4</sub> ·5H <sub>2</sub> O administration		
		Pre-treatment <sup>5)</sup>	Treatment period <sup>5)</sup>	Post-treatment <sup>5)</sup>
Feces <sup>2)</sup>	9	19±3 <sup>6)</sup>	202±39 <sup>6)</sup>	19±1 <sup>7)</sup>
Urine <sup>3)</sup>	3	0.01±0.00	0.01±0.00	0.01±0.00
Milk <sup>2)</sup>	3	0.38±0.09	0.53±0.10	0.28±0.09
Blood <sup>2)</sup>	2	4.8±0.4	5.2±0.3	5.1±0.7
Hair <sup>2)</sup>	1	7.4±0.1	34.5±15.4	12.2

1) administered 1 g of Cu as CuSO<sub>4</sub>·5H<sub>2</sub>O daily for 4 weeks. 2) ppm on dry matter basis. 3) ppm on wet matter basis. 4) obtained from a cow. 5) Each period consists of 4 weeks. 6) Mean±S.D. of collections of 2 cows. 7) Mean±S.D. of collections of a cow.

Table 15. Copper concentration in tissues of copper-treated and untreated cattle

Group	untreated <sup>1)</sup>					treated	
	1	2	3	4	5	9 <sup>2)</sup>	10 <sup>3)</sup>
Cattle No.							
Age ( mos. )	newborn	9	65	79	115	90	32
Body weight ( kg )	54	205	574	500	690	598	461
Liver	231. <sup>4)</sup>	144.	9.	170.	75.	368.	273.
Kidney	11.	22.	21.	18.	22.	19.	21.
Heart	15.9	20.0	15.2	15.6	16.4	15.6	17.7
Lung	8.6	5.5	5.5	8.7	4.6	6.7	8.4
Pancreas	---	4.1	2.0	4.4	2.8	3.5	3.4
Spleen	4.8	3.6	3.2	---	2.4	5.8	4.8
Gall bladder	---	7.5	---	---	1.4	1.4	13.1
Diaphragm	---	4.1	1.8	5.8	3.8	4.1	4.3
M. Biceps femoris	4.9	2.2	2.0	3.1	4.3	3.1	3.0
M. Longissimus thoracis	---	2.2	1.4	2.4	2.2	3.2	1.8
Rumen	---	5.6	3.3	3.5	3.6	4.8	9.6
Reticulum	---	4.0	---	---	5.7	4.9	6.0
Omasum	---	4.4	---	---	4.2	3.4	11.0
Abomasum	2.3	6.0	---	---	9.3	3.0	8.0
Small intestine	4.6	4.7	2.4	4.2	2.4	3.6	4.2
Large intestine	4.8	3.6	1.8	2.1	4.1	1.1	4.6
Uterus	---	2.7	---	---	2.1	3.0	3.2
Urinary bladder	---	1.8	---	---	2.3	2.8	1.4
Skin	---	4.0	---	---	0.3	1.3	1.3

1) 5 cattle of different ages from the 19 cattle shown in Fig. 3.

2) slaughtered 4 weeks after the termination of the administration.

3) slaughtered immediately after the administration. 4) ppm on dry matter basis.

#### Section 4. Effect of dietary Mn level on Mn nutrition of cattle

Manganese is essential for the functioning of several enzyme systems and usually combined with other compounds especially with proteins in soft tissues ( 142, 230 ). Also, compared with Mn concentration in feed, the animal body has a very low average Mn concentration which was 0.3 ppm in a dairy cattle ( 142 ).

Ruminants have developed a mechanism of homeostatic control enabling them to perform normally with varying intakes of Mn, even when the Mn content of feed varies widely ( 142, 230 ). In Japan, Mn nutrition has received little attention in either research or practical feeding of dairy cattle, since Mn content of Japanese pasture ( 152, 204 ) is almost above the 40 ppm dietary Mn requirement for dairy cattle ( NRC ( 149 ) ). However, it may be necessary to evaluate Mn nutrition in dairy cattle, since Mn deficiency with its typical symptoms of retarded growth, bone abnormalities, and reproductive disturbances has been reported in ruminants ( 142, 230 ). The objective of this section was, therefore, to clarify the effect of dietary Mn level on the Mn status of Holstein cattle.

#### Materials and Methods

##### 1. Investigation of Mn concentration in feed and tissues of cattle

The experiment was conducted as previously described ( Section 1. ).

##### 2. Effect of high Mn level in rations on Mn status in the body

Two lactating cows were fed 10 g of Mn as manganese sulfate mixed in their concentrate, daily for 4 weeks. One cow was slaughtered immediately after the administration, while the other one was slaughtered 4 weeks after the termination of the administration. The experiment was performed from 4 weeks before Mn administration to slaughter and consisted of 3

periods: pre-treatment, treatment, and post-treatment. Both cows were fed 4 kg concentrate, 1-2 kg beet pulp, and Italian ryegrass hay ad libitum. They received water and mineral salt as a block ad libitum. They were slaughtered at 65 and 79 months old, and 564 and 502 kg in body weight, respectively.

Feces were collected 9 times each period with grab sampling from the rectum, and urine and milk were collected 3 times each period. Blood and hair were collected just at the termination of each period; blood was obtained by jugular puncture, and hair was collected from the portion around the scapula. Feed samples were taken randomly. Tissues of treated cows were obtained immediately after slaughter. Five untreated cattle were chosen on the basis of age, and their tissues were obtained as described above.

### 3. Mn analysis of samples

Preparation of samples was conducted as previously described ( Section 2. ). Manganese concentration in tissues, feces, hair, and feed was determined by neutron activation analysis without ashing ( 106 ), but that in blood, milk, and urine was determined by atomic absorption spectrophotometry after digested samples were extracted with methyl isobutyl ketone ( 238 ). Each value, except for urine, is expressed on a dry matter basis. Statistical differences were evaluated by students t'test.

## Results

### 1. Investigation of Mn concentration in feed and tissues of cattle

Manganese concentration in feed at the station is shown in Table 16. Manganese concentration in Italian ryegrass and Tall oatgrass was 2-3 fold higher than that in concentrate and beet pulp. Manganese concentration in



water was below 0.01 ppm. From the figures for Mn concentration in feed and feed intake, it was assumed that Mn levels in total diets of dry, lactating, and fattening cows were 80-115, 65-95, and 50-60 ppm, respectively.

Manganese concentration in the liver, kidney, blood, and feces of cows at the station is shown in Table 17. There were no significant differences between groups in Mn concentration in the liver, kidney, and blood, but Mn concentration in the feces of dry cows was significantly higher than that of fattening cows. Also, Mn concentration in the feces of lactating cows was higher than for fattening cows, but not significantly different.

Manganese concentration in the liver and kidney of 19 cattle kept at the station is shown in Fig. 4. Manganese concentrations in the liver and kidney were 3.83-5.48 and 1.14-2.04 ppm, respectively in 2 newborn calves, but those in 1 month old calf were 9.73 and 1.96 ppm, respectively. Also, in 14 cows those values at 8.28-15.07 and 3.00-5.96 ppm were higher than those in newborn calves. Manganese concentration in the liver was higher than in the kidney, and that in the liver and kidney was not affected by age.

Manganese concentration in samples of cows and feed obtained from 10 dairy farms is shown in Table 18. Manganese concentration in roughage was higher than that in concentrate and by-product feed; that in rice straw was highest. Manganese concentration in the liver on dairy farms was slightly lower than that at the station, but that in the kidney and blood on dairy farms was almost similar to that at the station.

## 2. Effect of high Mn level in rations on Mn status in the body

There was a slight decrease in feed intake by one cow for the first week within the treatment period, but by the second week it returned to normal. However, feed intake of the other cow was not affected by Mn administration. Daily milk yields of treated cows were 7-11 and 11-12 kg,

respectively, and not affected by Mn administration. Manganese concentrations in concentrate, beet pulp, and Italian ryegrass were 51, 55, and 100 ppm, respectively. It was assumed that daily Mn intake and dietary Mn level in the pre-treatment and post-treatment periods were 1.0-1.3 g and 80 ppm, respectively, whereas those of the treatment period were 11 g and 750 ppm, respectively.

Manganese concentration in the feces, urine, milk, blood, and hair of 2 Mn-treated cows is shown in Table 19. Manganese concentration in the feces in the treatment period was about 7 fold higher than before or after treatment, but that in the urine in the treatment period was similar to before or after treatment. Manganese concentration in the milk, blood, and hair in the treatment period was about 2-5 fold higher than before or after treatment. Assuming that digestibility of dry matter and daily urine amounts were 65 % and 10 kg, respectively, daily Mn excretion in the feces, urine, and milk of the pre-treatment and post-treatment periods were 1.1, 0.0001, and 0.0004 g, respectively, whereas those of the treatment period were 8.0, 0.0001, and 0.0014 g, respectively.

Manganese concentration in the tissues of treated and untreated cattle is shown in Table 20. Manganese concentration in the tissues of a newborn calf was lower than for cows. Higher Mn concentration in the untreated cattle was obtained from the rumen, reticulum, omasum, abomasum, liver, skin, kidney, and pancreas. Manganese concentration in most tissues of the cow slaughtered 4 weeks after the termination of the administration was almost similar to that for untreated cattle, but that in the rumen, reticulum, and omasum was higher. Also, Mn concentration in the tissues of the other cow slaughtered immediately after the administration was higher than for untreated cattle; Mn concentration in the rumen, reticulum, and omasum was very high, and that in the rumen content was 676 ppm.

## Discussion

It is suggested that Mn intake was sufficient in this study, since dietary Mn level was almost above the 40 ppm of dietary Mn requirement for dairy cattle ( NRC ( 149 ) ). However, Mn concentration in concentrate and by-product feed was slightly low. ADAMS ( 2 ) reported that corn grain adapted from NRC publications contained an average of 5.7 ppm Mn and barley of 18.2 ppm Mn. Furthermore, a majority of corn silage produced in Ontario contained deficient amounts of Mn ( 22 ). It is suggested that there may be a possibility of Mn deficiency in ruminants, since concentrate in Japan consisted almost entirely of corn and barley and corn silage was used widely. Sufficient Mn, therefore, should be added up to at least 40 ppm Mn in the total ration if low Mn diets were used.

In ruminants Mn deficiency produces a number of striking symptoms in the fetus or neonate ( 61, 63, 113 ). ROJAS et al. ( 186 ) reported that six calves born from cows 16-17 ppm dietary Mn for a 12-month period had neonatal deformities. HOWES and DYER ( 67 ) reported that lower concentration of Mn was detected in newborn calf tissue when their dams received a lower level of Mn, and the newborn calf preferentially stored Mn in the liver. In this study tissue Mn concentration of newborn calves born from cows fed sufficient Mn was lower than for cows. HIDIROGLOU ( 63 ) indicates that knowledge of the intimate relationships between Mn and other minerals is just beginning to emerge, and a dietary deficiency of Mn in the fetus results in altered utilization of other mineral elements in the fetus. Thus, it is suggested that Mn transfer to the fetus across the placenta was prevented and low Mn concentration in the tissue of newborn calves was caused by the effect of mineral interactions rather than by the effect of Mn concentration in maternal tissue.

The absorption of ingested Mn is generally considered to be very low,

with 3-4 % being typical, and changes in absorption and endogenous fecal excretion of Mn are of major importance in the Mn homeostatic control mechanism ( 1, 24, 61, 86, 87, 95, 139, 140, 235 ). In this study Mn concentration in the body of the cows was not affected by dietary Mn level, although dietary Mn level in dry and lactating cows was higher than in fattening cows. Also, most of dietary Mn may be excreted in the feces, because Mn concentration in the feces increased with the increase in dietary Mn level. It is suggested that tissue Mn concentration in cattle fed sufficient Mn was usually maintained within narrow limits by means of changes in absorption and endogenous fecal excretion of Mn.

Tissue Mn concentration in ruminants increase with extreme increases in dietary Mn intake ( 60, 61, 95, 139, 142, 230 ). This fact coincided with this study, since Mn concentration in tissues, milk, blood, and hair of Mn-treated cows was higher than for untreated cattle. However, most of Mn administered may be excreted in the feces by means of changes in absorption and endogenous fecal excretion of Mn, since Mn concentration in the feces of Mn-treated cows was much higher than before or after treatment, but not in the urine.

It is estimated by the NRC ( 149 ) that dairy cattle can tolerate 1000 ppm dietary Mn without adverse effect. CUNNINGHAM et al. ( 34 ) reported that weight gains and feed consumption of Holstein calves were not adversely affected by 820 ppm supplemental Mn, but decreased by 2460 ppm. Feed intake and daily milk yield of Mn-treated cows in this study were not affected by the administration of 750 ppm in the diet, except for slight decrease in feed intake at the start of Mn administration. Also, dairy cattle may be not affected by high Mn diets in Japan, since Mn concentration in feed was below 1000 ppm in this study and an earlier report ( 204 ). However, HIDIROGLOU ( 61 ) indicates that a variety of metabolic interactions are known to exist among trace elements, and

a dietary deficiency of one trace element may result not only in a concentration change in that element in the animal body but also in accompanying changes in other elements because of biological interaction. It is, therefore, suggested that mineral interaction with mineral nutrition should be taken into account when using variable feeds in Japanese farms, although Mn intake was sufficient in this study.

Table 16. Manganese concentration in feed obtained from  
Kyushu Natl. Agric. Exp. Stn.

Feed	Number of samples	Mn concentration <sup>1)</sup>
Concentrate	4	40 <u>±</u> 23 <sup>2)</sup>
Beet pulp	2	56 <u>±</u> 1
Italian ryegrass ( hay )	2	84 <u>±</u> 23
Italian ryegrass ( low moisture silage )	2	121 <u>±</u> 4
Tall oatgrass ( hay )	2	94 <u>±</u> 5

1) ppm on dry matter basis. 2) Mean±S.D.

Table 17. Manganese concentration in liver, kidney, blood and feces of Holstein cows kept at Kyushu Natl. Agric. Exp. Stn.

	Dry	Lactating	Fattening
Number of cows	6	4	4
Dietary Mn level <sup>1)</sup>	80-115 <sup>2)</sup>	65-95	50-60
Liver <sup>1)</sup>	11.17±1.56 <sup>a)3)</sup>	11.42±2.49 <sup>a)</sup>	11.17±1.23 <sup>a)</sup>
Kidney <sup>1)</sup>	4.84±1.04 <sup>a)</sup>	3.54±0.47 <sup>a)</sup>	3.62±0.60 <sup>a)</sup>
Blood <sup>1)</sup>	0.19±0.03 <sup>a)</sup>	0.20±0.07 <sup>a)</sup>	0.23±0.06 <sup>a)</sup>
Feces <sup>1)</sup>	276±51 <sup>a)</sup>	289±64 <sup>a,b)</sup>	201±38 <sup>b)</sup>

a and b: Values in the same line with different superscripts are significantly different (  $P < 0.05$  ) 1) ppm on dry matter basis.

2) Range of dietary Mn level. 3) Mean±S.D.

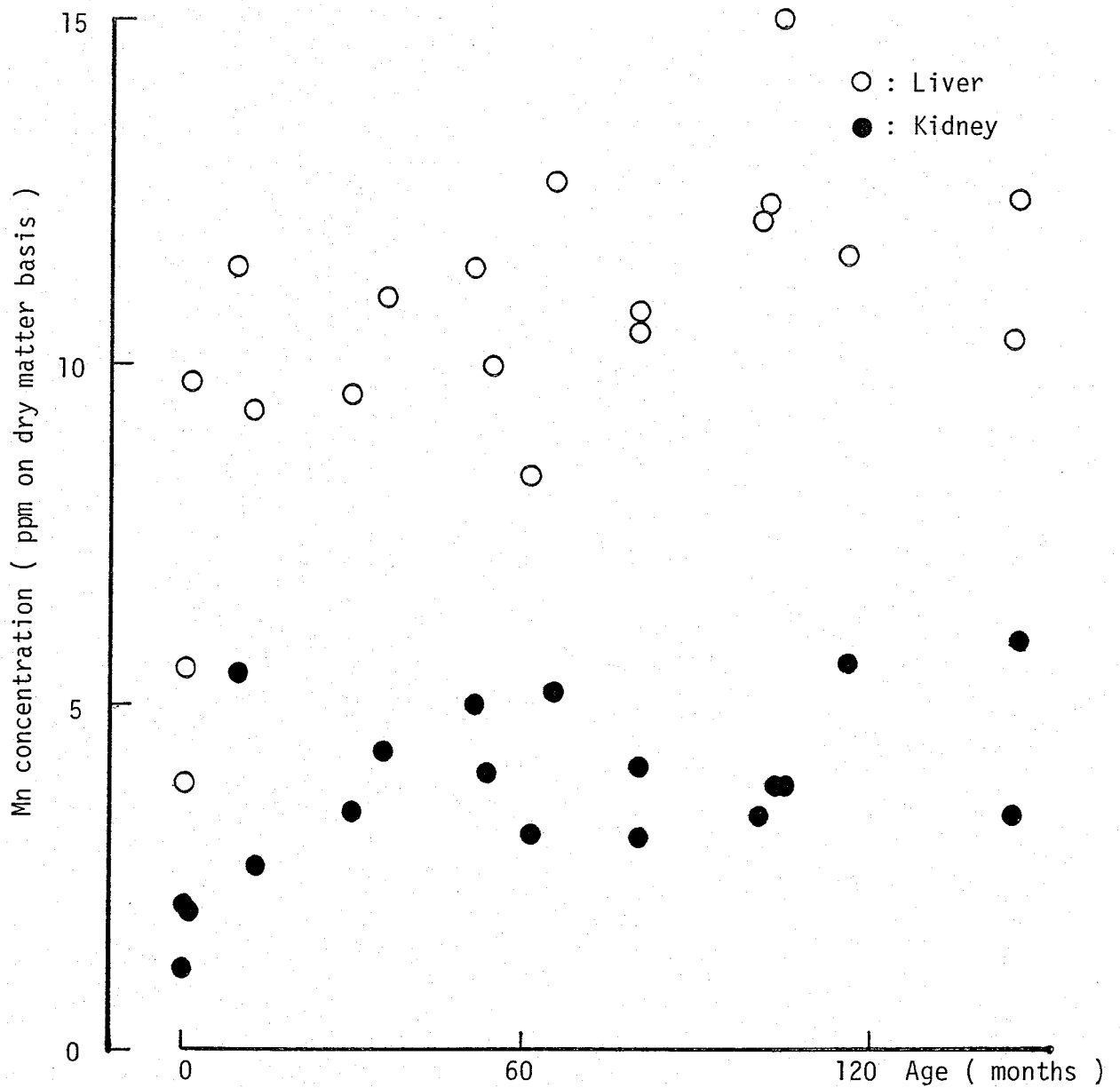


Fig. 4. Manganese concentration in liver and kidney of Holstein cattle kept at Kyushu Natl. Agric. Exp. Stn.



Table 18. Manganese concentration in samples of Holstein cows  
and feed obtained from 10 dairy farms in Kumamoto prefecture

Sample	Number of samples	Mn concentration <sup>1)</sup>	
		Mean±S.D.	Range
Cow			
Liver	16	7.98±1.07	6.26-10.01
Kidney	18	4.68±0.94	3.48-7.16
Feces	18	244±85	136-437
Feed			
Concentrate	7	33±3	30-39
Corn ( soiling and silage )	6	82±76	33-233
Italian ryegrass ( hay )	1	108	
Mixed grass ( hay )	1	102	
Wild grass ( hay )	1	159	
Rice straw	4	332±132	236-523
Citrus pulp	4	12±4	7-16
Tofu pulp	1	18	
Whisky by-product	1	44	

1) ppm on dry matter basis. 2) Commercial formula feed

Table 19. Manganese concentration in feces, urine, milk, blood, and hair of 2 manganese-treated cows<sup>1)</sup>

Item	Number of collections <sup>4)</sup>	MnSO <sub>4</sub> ·4.9H <sub>2</sub> O administration		
		Pre-treatment <sup>5)</sup>	Treatment period <sup>5)</sup>	Post-treatment <sup>5)</sup>
Feces <sup>2)</sup>	9	224±31 <sup>6)</sup>	1564±228 <sup>6)</sup>	198±22 <sup>7)</sup>
Urine <sup>3)</sup>	3	0.01±0.00	0.01±0.00	0.01±0.00
Milk <sup>2)</sup>	3	0.31±0.06	1.13±0.69	0.29±0.04
Blood <sup>2)</sup>	1	0.20±0.01	0.46±0.01	0.32
Hair <sup>2)</sup>	1	22.1±2.1	97.6±25.9	24.6

1) administered 10 g of Mn as MnSO<sub>4</sub>·4.9H<sub>2</sub>O daily for 4 weeks. 2) ppm on dry matter basis. 3) ppm on wet matter basis. 4) obtained from a cow. 5) Each period consists of 4 weeks. 6) Mean±S.D. of collections of 2 cows. 7) Mean±S.D. of collections of a cow.

Table 20. Manganese concentration in tissues of Manganese-treated and untreated cattle

Group	untreated <sup>1)</sup>					treated	
	1	2	3	4	5	11 <sup>2)</sup>	12 <sup>3)</sup>
Cattle No.							
Age ( mos. )	newborn	9	65	79	115	65	79
Body weight ( kg )	54	205	574	500	690	564	502
<hr/>							
Liver	5.48 <sup>4)</sup>	11.38	12.60	10.40	11.54	10.33	18.50
Kidney	2.04	5.42	5.19	3.00	5.60	2.60	8.90
Heart	0.66	1.87	2.09	2.04	1.29	2.17	2.76
Lung	0.38	0.91	1.18	1.72	1.05	2.44	4.56
Pancreas	---	3.96	8.68	7.00	5.70	5.53	11.40
Spleen	0.76	0.63	1.50	---	1.62	1.42	10.72
Gall bladder	---	1.14	---	---	0.78	0.49	7.18
Diaphragm	---	0.40	0.35	0.68	0.61	0.65	6.94
M. Biceps femoris	0.54	0.51	0.42	0.52	0.45	0.86	3.82
M. Longissimus thoracis	---	0.63	0.40	0.51	0.27	0.45	4.51
Rumen	---	12.61	21.58	15.92	8.24	67.13	1059.
Reticulum	---	12.98	---	---	17.86	51.47	1276.
Omasum	---	20.94	---	---	21.79	66.78	296.
Abomasum	0.95	11.14	---	---	8.06	10.86	22.58
Small intestine	3.90	4.84	7.66	4.01	3.88	1.70	13.18
Large intestine	4.55	5.68	6.33	2.16	2.82	1.45	8.17
Uterus	---	1.67	---	---	0.68	2.73	5.48
Urinary bladder	---	2.66	---	---	1.51	3.34	7.77
Skin	---	12.79	---	---	6.85	7.38	17.97

1) 5 cattle of different ages from the 19 cattle shown in Fig. 4.

2) slaughtered 4 weeks after the termination of the administration.

3) slaughtered immediately after the administration. 4) ppm on dry matter basis.

## Section 5. Effect of dietary Se level on Se nutrition of cattle

Selenium is necessary for growth and fertility in animals and for the prevention of various disease conditions which show a variable response to vitamin E ( 3, 142, 149, 230 ). Also, Se is an essential component of glutathion peroxidase which aids in protecting cellular and subcellular membranes from oxidative damage ( 142, 230 ).

The best known syndrome of severe Se deficiency in cattle is " white muscle disease ", nutritional muscular dystrophy, in young calves ( 149 ). Also, Se toxicity is classified as either acute, often called blind staggers, or chronic, which frequently is termed alkali disease ( 149 ). Although long recognized as a toxic element, Se deficiency in ruminants is much widespread and economically important than the toxicity ( 142, 230 ). Since Se content in Japanese pasture ( 14 ) is almost below the 0.1 ppm dietary Se requirement of dairy cattle, Se deficiency in the practical feeding of the cattle has become recognized as a major problem in Japan. The objective of this section was, therefore, to clarify the effect of dietary Se level on the Se status of Holstein cattle.

### Materials and Methods

#### 1. Investigation of Se concentration in feed and tissues of cattle

The experiment was conducted as previously described ( Section 1. ).

#### 2. Effect of high Se level in rations on Se status in the body

Two lactating cows were fed 0.1g of Se as sodium selenate mixed in their concentrate, daily for 4 weeks. One cow was slaughtered immediately after the administration, while the other one was slaughtered 4 weeks after the termination of the administration. The experiment was performed from 4 weeks before Se administration to slaughter and consisted of 3

periods: pre-treatment, treatment, and post-treatment. Both cows were fed 4-6 kg concentrate, 1-2 kg beet pulp, and Italian ryegrass soiling, hay, and low moisture silage ad libitum. They received water and mineral salt as a block ad libitum. They were slaughtered at 110 and 34 months old, and 574 and 462 kg in body weight, respectively.

Feces were collected 2 times each period with grab sampling from the rectum, and urine and milk were collected 2 times each period. Blood and hair were collected just at the termination of each period; blood was obtained by jugular puncture, and hair was collected from the portion around the scapula. Feed samples were taken randomly. Tissues of treated cows were obtained immediately after slaughter. Five untreated cattle were chosen on the basis of age, and their tissues were obtained as described above.

### 3. Se analysis of samples

Preparation of samples was conducted as previously described ( Section 2. ). Samples were digested in nitric-perchloric acid and Se concentration was determined by fluorometric analysis after extraction with cyclohexane ( 234 ). Each value, except for urine, is expressed on a dry matter basis. Statistical differences were evaluated by students t'test.

## Results

### 1. Investigation of Se concentration in feed and tissues of cattle

Selenium concentration in feed at the station is shown in Table 21. Selenium concentration in Italian ryegrass and Tall oatgrass was 5-8 fold lower than that in concentrate and beet pulp. Selenium concentration in water was below 0.01 ppm. From the figures for Se concentration in feed and feed intake, it was assumed that Se levels in total diets of dry, lactating, and fattening cows were 0.03-0.05, 0.08-0.11, and 0.10-0.17

ppm, respectively.

Selenium concentration in the liver, kidney, blood, and feces of cows at the station is shown in Table 22. Selenium concentration in the liver, blood, and feces of fattening and lactating cows was significantly higher than for dry cows, and that in the liver and blood of fattening cows was highest. However, there were no significant differences between groups in Se concentration in the kidney.

Selenium concentration in the liver and kidney of 19 cattle kept at the station is shown in Fig. 5. Selenium concentration in the liver and kidney were 1.26–1.74 and 1.81–2.92 ppm respectively in 2 newborn and one 1 month old calves, but those in 2 heifers were 1.10–1.11 and 4.42–5.38 ppm, respectively. Also, those in 14 cows were 0.43–1.42 and 3.54–6.41 ppm, respectively. Selenium concentration in the liver of calves was higher than for cows, but not in the kidney. Selenium concentration in the kidney was higher than in the liver, and that in the liver and kidney of cows was not affected by age.

Selenium concentration in samples of cows and feed obtained from 10 dairy farms is shown in Table 23. Selenium concentration in corn, Italian ryegrass, rice straw, and citrus pulp was lower than that in concentrate, mixed grass, wild grass, tofu pulp, and whisky by-product. Selenium concentration in the liver, kidney, and feces on dairy farms was almost similar to that of lactating and fattening cows at the station.

## 2. Effect of high Se level in rations on Se status in the body

There was a decrease in feed intake and rapid salivation for one cow during the first week within the treatment period, but by the second week it returned to normal according to decreased dose of 20 % of Se administration. However, there was a swelling of the servix and sore feet at the 10 days and continued for a week, but thereafter it returned to normal. Feed intake of the other cow was not affected by Se administration.

Daily milk yields of treated cows were 16–20 and 8–10 kg respectively, and not affected by Se administration. Selenium concentrations in concentrate, beet pulp, and Italian ryegrass soiling, hay, and low moisture silage were 0.28, 0.18, 0.02, 0.03, and 0.07 ppm, respectively. It was assumed that daily Se intake and dietary Se level in the pre-treatment and post-treatment periods were 2 mg and 0.14 ppm, respectively, whereas those of the treatment period were 100 mg and 6.5 ppm, respectively.

Selenium concentration in the feces, urine, milk, blood, and hair of 2 Se-treated cows is shown in Table 24. Selenium concentration in the feces in the treatment periods was about 16–18 fold higher than before or after treatment, and that in the urine and milk was about 12–16 and 3–4 fold higher, respectively. Also, Se concentration in the blood in the treatment period was about 7 fold higher than before treatment, and that in the post-treatment period was about 4 fold higher than before treatment. Furthermore, Se concentration in the hair in the treatment period was about 3 fold higher than before treatment, but that in the post-treatment period was about 8 fold higher. Assuming that digestibility of dry matter and daily urine amounts were 65 % and 10 kg, respectively, daily Se excretion in the feces, urine, and milk of the pre-treatment and post-treatment periods were 1.7, 0.6, 0.5 mg, respectively, whereas those of the treatment period were 30, 8, and 1.7 mg, respectively.

Selenium concentration in the tissues of treated and untreated cattle is shown in Table 25. Higher Se concentration in the untreated cattle was obtained from the kidney, liver, heart, and pancreas. Selenium concentration in most tissues of the cow slaughtered 4 weeks after the termination of the administration was slightly higher than for untreated cattle, and that in the liver was considerably higher. Also, Se concentration in the tissues of the other cow slaughtered immediately after the administration was considerably higher than for untreated

cattle, and that in the liver was highest.

## Discussion

The requirement for Se by ruminants is approximately 0.1 ppm, depending upon the chemical form of Se and the levels of interfering or enhancing factors in the diets, including vitamin E, S, lipids, proteins, amino acids, and several macro element ( NRC ( 149 ) ). Since Se concentration in roughage was almost below 0.1 ppm, Se intake by the cattle fed mainly roughage in this study was marginally inadequate. One of the most important and best known interactions in nutrition is that between Se and vitamin E, and higher levels of vitamin E reduce the requirement for Se and vice versa ( 3, 50, 89, 90, 145, 192, 197, 230 ). Although vitamin E was not determined in this study, there may be the possibility of a borderline Se deficiency for the cattle fed rations of low Se content as roughage in Japan, because Se content in roughage was almost below 0.1 ppm in this study and earlier reports ( 14, 166 ).

Selenium concentration in the tissues is reflected by the level of dietary Se, and Se content of certain tissues, of which kidney and liver Se are the best indicators, declines with a deficiency ( 5, 10, 12, 23, 29, 51, 66, 120, 178-180, 230, 237 ). Thus, Se values above 0.1 and 1.0 ppm on a wet matter basis for liver and kidney cortex are normal for sheep ( 12 ). Compared with Se concentrations in the liver and kidney in this study on the basis of a wet matter basis ( 185 ), those were above 0.1 and 1.0 ppm. However, Se concentration in the liver, blood, and feces was affected by dietary Se level. It is, therefore, suggested that Se deficiency in the cattle may occur after long periods of feeding low Se diets, since Se concentration in the body decreased with reduced dietary Se.



The lowest Se toxic level is approximately 3-5 ppm, depending upon the proteins, S, and arsenic levels of the diets and the chemical form of Se ( 45, 114, 149, 175, 176 ). Selenium toxicity of cattle may not occur in Japan except in cases of Se contamination in soil and plants, since Se concentration in feed in this study and earlier papers ( 14, 166 ) was below 3 ppm. However, chronic Se toxicity of the cow occurred by the administration of 6.5 ppm Se in the diets, because it includes loss of appetite, loss of vitality, and sore feet. Therefore, it is necessary to pay attention to Se contamination in feed, since it may be the possibility of the excesses of Se supplementation in feed because of the narrow range between the requirement and toxic level.

The main target organ of Se toxicity, the liver, can apparently rid itself of excess Se, and following injections of Se, retained Se is lost from the tissues at first rapidly and then more slowly ( 230 ). This was indicated in this study, since Se concentration in the liver of Se-treated cows was highest among the tissues and that in the feces and urine of treatment period was higher than before or after treatment. Also, It has been reported that Se is excreted through the feces, urine, and respiration, and fecal excretion of ingested Se is generally greater than urinary excretion in ruminants ( 230 ). Furthermore, most of Se in the feces consists of unabsorbed Se, and most of endogenous excretion of Se is through urine ( 230 ). In this study, most of Se administered was excreted in the feces and urine, and slightly secreted in the milk. However, it is suggested that some Se remained for long periods in the body, since Se concentration in the liver, blood, and hair of the cow slaughtered 4 weeks after the termination of the administration was greater than for untreated cattle in this study.

Liver Se concentration of newborn calves born from cows fed inadequate Se in this study was higher than for cows, but not in the other tissues.

It is apparent that Se is transmitted through the placenta to the fetus and liver Se may be important in Se metabolism of calves. In recent years, Se nutrition in ruminants was usually studied in Japan ( 14, 80, 81, 107, 109, 166, 200 ). However, further study may be needed on Se nutrition, since Se deficiency in grazing cattle has become recognized as a major practical problem in Japan.

Table 21. Selenium concentration in feed obtained from  
Kyushu Natl. Agric. Exp. Stn.

Feed	Number of samples	Se concentration <sup>1)</sup>
Concentrate	4	0.16±0.02 <sup>1)</sup>
Beet pulp	2	0.14±0.13
Italian ryegrass ( hay )	2	0.03±0.00
Italian ryegrass ( low moisture silage )	2	0.02±0.01
Tall oatgrass ( hay )	2	0.02±0.00

1) ppm on dry matter basis. 2) Mean±S.D.

Table 22. Selenium concentration in liver, kidney, blood and feces of Holstein cows kept at Kyushu Natl. Agric. Exp. Stn.

	Dry	Lactating	Fattening
Number of cows	6	4	4
Dietary Se level <sup>1)</sup>	0.03-0.05 <sup>2)</sup>	0.08-0.11	0.10-0.17
Liver <sup>1)</sup>	0.67±0.14 <sup>a)3)</sup>	1.15±0.20 <sup>b)</sup>	1.36±0.03 <sup>b)</sup>
Kidney <sup>1)</sup>	5.03±0.91 <sup>a)</sup>	5.42±0.67 <sup>a)</sup>	5.19±0.37 <sup>a)</sup>
Blood <sup>1)</sup>	0.31±0.07 <sup>a)</sup>	0.47±0.09 <sup>b)</sup>	0.58±0.07 <sup>b)</sup>
Feces <sup>1)</sup>	0.11±0.02 <sup>a)</sup>	0.25±0.08 <sup>b)</sup>	0.21±0.01 <sup>b)</sup>

a and b: Values in the same line with different superscripts are significantly different (  $P < 0.05$  ) 1) ppm on dry matter basis.

2) Range of dietary Se level. 3) Mean±S.D.

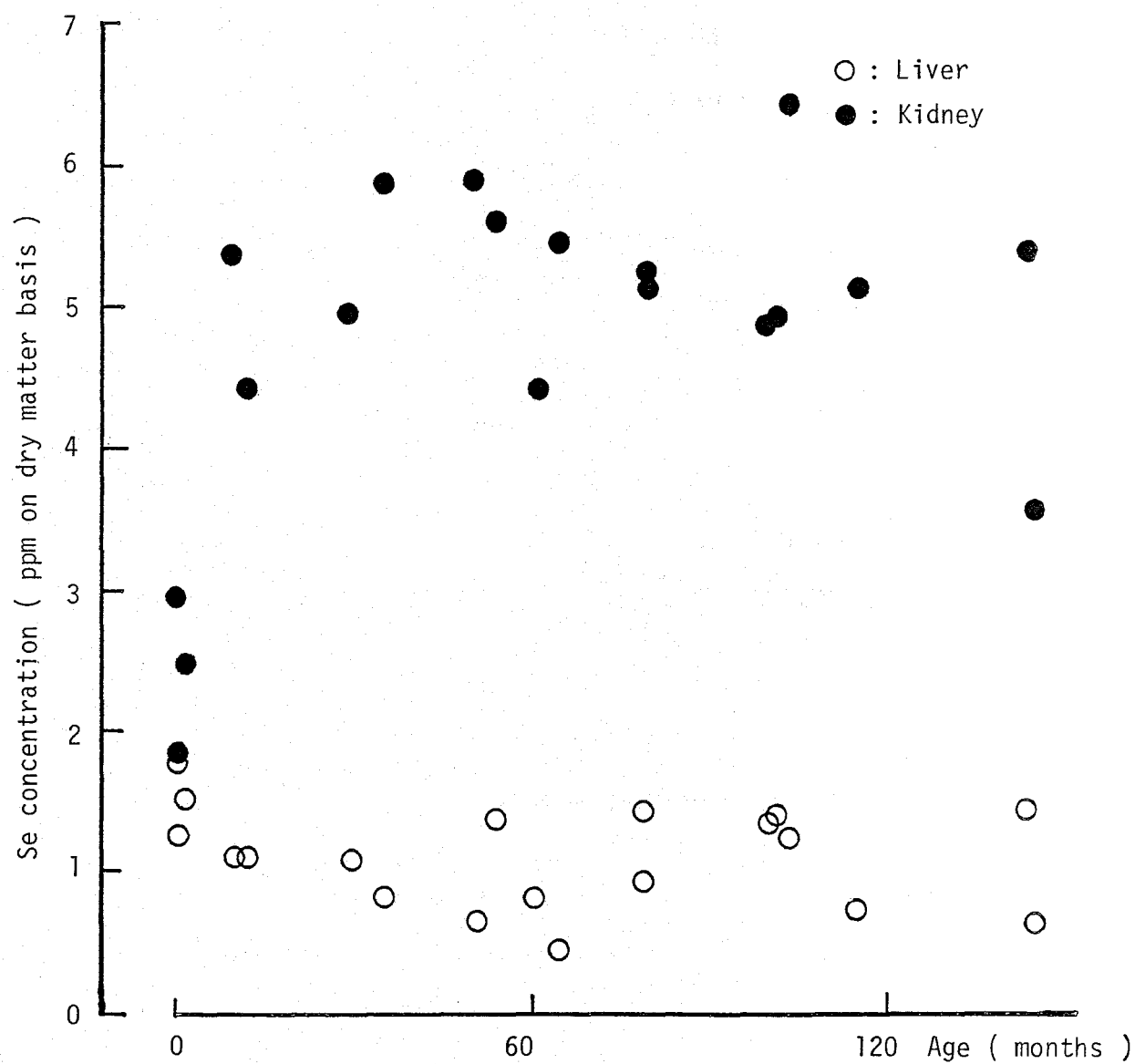


Fig. 5. Selenium concentration in liver and kidney of Holstein cattle kept at Kyushu Natl. Agric. Exp. Stn.

Table 23. Selenium concentration in samples of Holstein cows and feed obtained from 10 dairy farms in Kumamoto prefecture

Sample	Number of samples	Se concentration <sup>1)</sup>	
		Mean±S.D.	Range
Cow			
Liver	16	1.14±0.14	0.82-1.40
Kidney	18	6.18±0.54	5.36-7.17
Feces	18	0.36±0.10	0.25-0.62
Feed			
Concentrate <sup>2)</sup>	7	0.21±0.06	0.15-0.32
Corn	6	0.07±0.06	0.01-0.19
( soiling and silage )			
Italian ryegrass	1	0.01	
( hay )			
Mixed grass	1	0.22	
( hay )			
Wild grass	1	0.14	
( hay )			
Rice straw	4	0.03±0.02	0.01-0.06
Citrus pulp	4	0.02±0.01	0.01-0.03
Tofu pulp	1	0.11	
Whisky by-product	1	0.29	

1) ppm on dry matter basis. 2) Commercial formula feed

Table 24. Selenium concentration in feces, urine, milk, blood, and hair of 2 selenium-treated cows<sup>1)</sup>

Item	Number of collections <sup>4)</sup>	Na <sub>2</sub> SeO <sub>4</sub> administration		
		Pre-treatment <sup>5)</sup>	Treatment period <sup>5)</sup>	Post-treatment <sup>5)</sup>
Feces <sup>2)</sup>	2	0.33±0.05 <sup>6)</sup>	5.86±0.85 <sup>6)</sup>	0.36±0.01 <sup>7)</sup>
Urine <sup>3)</sup>	2	0.07±0.03	0.81±0.45	0.05±0.01
Milk <sup>2)</sup>	2	0.25±0.05	0.93±0.16	0.31±0.02
Blood <sup>2)</sup>	1	0.67±0.06	4.86±1.00	2.39
Hair <sup>2)</sup>	1	0.60±0.07	1.84±0.66	4.69

1) administered 0.1 g of Se as Na<sub>2</sub>SeO<sub>4</sub> daily for 4 weeks. 2) ppm on dry matter basis. 3) ppm on wet matter basis. 4) obtained from a cow. 5) Each period consists of 4 weeks. 6) Mean±S.D. of collections of 2 cows. 7) Mean±S.D. of collections of a cow.

Table 25. Selenium concentration in tissues of selenium-treated and untreated cattle

Group	untreated <sup>1)</sup>					treated	
Cattle No.	1	2	3	4	5	13 <sup>2)</sup>	14 <sup>3)</sup>
Age ( mos. )	newborn	9	65	79	115	110	34
Body weight ( kg )	54	205	574	500	690	574	462
Liver	1.26 <sup>1)</sup>	1.10	0.43	1.42	0.70	2.74	17.84
Kidney	2.92	5.38	5.44	5.14	5.10	5.46	10.94
Heart	0.62	0.81	0.68	1.13	0.70	1.27	2.20
Lung	0.69	0.67	0.31	0.50	0.44	1.18	2.06
Pancreas	---	1.39	0.56	1.14	0.95	1.37	2.69
Spleen	0.84	0.62	0.25	---	0.58	1.22	2.59
Gall bladder	---	0.35	---	---	0.38	0.48	0.55
Diaphragm	---	0.49	0.30	0.46	0.33	0.61	1.03
M. Biceps femoris	0.32	0.51	0.29	0.55	0.44	---	---
M. Longissimus thoracis	---	0.54	0.28	0.57	0.44	---	---
Rumen	---	0.53	0.26	0.46	0.43	0.82	2.45
Reticulum	---	0.66	---	---	0.31	0.99	2.38
Omasum	---	0.52	---	---	0.40	0.93	2.72
Abomasum	0.46	0.44	---	---	0.55	0.73	1.63
Small intestine	0.68	0.95	0.53	0.80	0.95	1.07	2.74
Large intestine	0.41	0.69	0.36	0.48	0.65	1.01	2.29
Uterus	---	0.55	---	---	0.63	0.90	1.63
Urinary bladder	---	0.50	---	---	0.62	0.85	1.68
Skin	---	0.35	---	---	0.22	0.64	1.25

1) 5 cattle of different ages from the 19 cattle shown in Fig. 5.

2) slaughtered 4 weeks after the termination of the administration.

3) slaughtered immediately after the administration. 4) ppm on dry matter basis.



## Section 6. Effect of dietary Mo level on Mo nutrition of cattle

Molybdenum is an indispensable component of the flavoprotein enzyme, xanthine oxidase found in milk and widely distributed in animal tissue ( 142, 230 ). The absolute requirement of dairy cattle for Mo is very low, and Mo deficiency probably would not occur under practical conditions ( 3, 142, 149, 230 ). However, cattle are less tolerant of high Mo than other farm animals ( 142, 230 ). Thus, Mo toxicity is an important practical problem in grazing cattle in several areas of the world ( 3, 142, 149, 230 ).

Since Mo content in Japanese pasture ( 152, 206 ) is never above the 6 ppm dietary Mo toxicity level for dairy cattle ( NRC ( 149 )), Mo toxicity may not appear to be the major problem in the practical feeding of dairy cattle in Japan. However, Mo and Cu are antagonistic to each other in the animal body ( 143, 230 ), and Mo toxicity or Cu deficiency in ruminants has been reported in Japan ( 13, 52, 55, 163, 226 ). The objective of this section was, therefore, to clarify the effect of dietary Mo level on the Mo status of Holstein cattle.

### Materials and Methods

#### 1. Investigation of Mo concentration in feed and tissues of cattle

The experiment was conducted as previously described ( Section 1. ).

#### 2. Effect of high Mo level in rations on Mo status in the body

Two lactating cows were fed 0.1 g of Mo as ammonium molybdate mixed in their concentrate, daily for 4 weeks. One cow was slaughtered immediately after the administration, while the other one was slaughtered 4 weeks after the termination of the administration. The experiment was performed from 4 weeks before Mo administration to slaughter and consisted of 3

periods: pre-treatment, treatment, and post-treatment. Both cows were fed 6 kg concentrate, 2 kg beet pulp, and Italian ryegrass hay ad libitum. They received water and mineral salt as a block ad libitum. They were slaughtered at 29 and 42 months old, and 446 and 512 kg in body weight, respectively.

Feces were collected 9 times each period with grab sampling from the rectum, and urine and milk were collected 3 times each period. Blood samples were obtained 2 times each period by jugular puncture. Hair was collected just at the termination of each period from the portion around the scapula. Feed samples were taken randomly. Tissues of treated cows were obtained immediately after slaughter. Five untreated cattle were chosen on the basis of age, and their tissues were obtained as described above.

### 3. Mo analysis of samples

Preparation of samples was conducted as previously described ( Section 2. ). Samples were digested in nitric-perchloric acid and Mo concentration was determined by graphite furnace atomic absorption spectrophotometry. Each value, except for urine, is expressed on a dry matter basis. Statistical differences were evaluated by students t'test.

## Results

### 1. Investigation of Mo concentration in feed and tissues of cattle

Molybdenum concentration in feed at the station is shown in Table 26. Molybdenum concentration in Italian ryegrass was slightly higher than that in concentrate. Molybdenum concentration in water was below 0.01 ppm. From the figures for Mo concentration in feed and feed intake, it was assumed that Mo levels in total diets of dry, lactating, and fattening cows were 0.81-0.96, 0.75-0.88, and 0.62-0.63 ppm, respectively.

Molybdenum concentration in the liver, kidney, blood, and feces of cows

at the station is shown in Table 27. There were no significant differences between groups in Mo concentration in the liver, kidney, blood, and feces.

Molybdenum concentration in the liver and kidney of 19 cattle kept at the station is shown in Fig. 6. Molybdenum concentration in the liver and kidney were 2.86–3.82 and 1.54–1.61 ppm respectively in 2 newborn calves, but those in 1 month old calf were 4.65 and 2.10 ppm, respectively. Also, those in 14 cows were 4.06–5.40 and 1.78–2.72 ppm, respectively.

Molybdenum concentration in the liver of calves was higher than for cows. Also, Mo concentration in the liver was higher than in the kidney, and that in the liver and kidney of cows was not affected by age.

Molybdenum concentration in samples of cows and feed obtained from 10 dairy farms are shown in Table 28. Molybdenum concentration in roughage was almost similar to concentrate, and that in feed was below 1.2 ppm. Also, Mo concentration in the liver, kidney, and feces on dairy farms was almost similar to that at the station.

## 2. Effect of high Mo level in rations on Mo status in the body

Feed intake of 2 cows was not decreased by Mo administration. Daily milk yields of treated cows were 16–20 and 15–20 kg, respectively, and not affected by Mo administration. Molybdenum concentration in concentrate, beet pulp, and Italian ryegrass hay were 0.63, 0.69, and 0.52 ppm, respectively. It was assumed that daily Mo intake and dietary Mo level in the pre-treatment and post-treatment periods were 10 mg and 0.55 ppm, respectively, and those in the treatment period were 110 mg and 6.1 ppm, respectively.

Molybdenum concentration in the feces, urine, milk, blood, and hair of 2 treated cows is shown in Table 29. Molybdenum concentration in the feces in the treatment period was about 13 fold higher than before or after treatment, and that in the urine was about 11–17 fold higher. Molybdenum concentration in the milk, blood, and hair in the treatment period was

about 2-4 fold higher than before or after treatment. Assuming that digestibility of dry matter and daily urine amounts were 65 % and 10 kg, respectively, daily Mo excretion in the feces, urine, and milk of the pre-treatment and post-treatment periods were 7, 0.3, and 0.9 mg, respectively, whereas those of the treatment period were 97, 3.4, and 1.3 mg, respectively.

Molybdenum concentration in the tissues of treated and untreated cattle is shown in Table 30. Higher Mo concentration in the untreated cattle was obtained from the spleen, liver, kidney, and lung. Molybdenum concentration in most tissues of the cow slaughtered 4 weeks after the termination of the administration was almost similar to that for untreated cattle. However, Mo concentration in most tissues of the cow slaughtered immediately after the administration was higher than for untreated cattle; that in the spleen and liver was very high, and that in the rumen content was 16.2 pm.

## Discussion

Although the absolute requirement of dairy cattle for Mo has not been determined, it is believed to be very low; perhaps no more than 1 ppm ( 142, 149 ). However, cattle are the least tolerant of high Mo among farm animals ( 68, 98, 142, 230 ), and the maximum amount of Mo that could normally be consumed without toxicity is about 6 ppm in the dry matter of the diet ( NRC ( 149 ) ). Since Mo concentration in feed was below 1.2 ppm, Mo intake by the cattle was adequate in this study. However, Mo toxicity is an important problem in many areas of the world, although Mo deficiency has never been developed or observed in cattle ( 3, 142, 149, 230 ). It is, therefore, necessary to be attention to Mo contamination in feed because Mo toxicity in ruminants has been reported in Japan ( 13, 52, 55,

163, 226 ), although Mo concentration in feed was very low in this study and an earlier paper ( 206 ).

Molybdenum concentration in the liver, kidney, blood, and feces of the cows was not affected by dietary Mo level, and feed intake and daily milk yields were not affected by the administration of 6.1 ppm Mo in the diet in this study. However, Mo and Cu are antagonistic to each other in the animal body, and high Mo levels interfere with Cu metabolism ( 35-37, 116, 117, 144, 168, 202, 230, 232 ). It seems likely that Cu deficiency may occur after long periods of feeding low Cu diets in Japan, since Cu concentration in the liver decreased with reduced dietary Cu and low Cu concentration in the liver was regarded as Cu deficiency in this study. However, it is not apparent whether Cu deficiency was affected by dietary Mo level, since Mo level in feed was very low and Cu deficiency according to Mo administration did not occur in this study.

Molybdenum concentration in the feces, urine, milk, blood, and hair of treatment period was higher than before or after treatment, and higher Mo concentration of Mo-treated cows was obtained from most tissues. It was suggested that most of Mo administered was excreted in the feces and urine, but some Mo accumulated in the body. Furthermore, Mo concentration in the liver of newborn calves was lower than for cows, but Cu concentration was higher. It may be, therefore, necessary to pay attention to the interaction between Mo and Cu, since the interaction between Mo and Cu have not been studied sufficiently with dairy cattle.

Table 26. Molybdenum concentration in feed obtained from  
Kyushu Natl. Agric. Exp. Stn.

Feed	Number of samples	Mo concentration <sup>1)</sup>
Concentrate	4	0.62±0.11 <sup>2)</sup>
Beet pulp	2	0.80±0.02
Italian ryegrass ( hay )	2	0.84±0.01
Italian ryegrass ( low moisture silage )	2	1.00±0.26
Tall oatgrass ( hay )	2	0.65±0.14

1) ppm on dry matter basis. 2) Mean±S.D.

Table 27. Molybdenum concentration in liver, kidney, blood and feces of Holstein cows kept at Kyushu Natl. Agric. Exp. Stn.

	Dry	Lactating	Fattening
Number of cows	6	4	4
Dietary Mo level <sup>1)</sup>	0.81-0.96 <sup>2)</sup>	0.75-0.88	0.62-0.63
Liver <sup>1)a)</sup>	4.77±0.42 <sup>3)</sup>	4.54±0.47	4.51±0.32
Kidney <sup>1)a)</sup>	2.47±0.24	2.14±0.37	2.50±0.28
Blood <sup>1)a)</sup>	0.11±0.02	0.10±0.01	0.12±0.01
Feces <sup>1)a)</sup>	1.47±0.16	1.42±0.21	1.42±0.14

a: Values in the same line are not significantly different (  $P>0.05$  )

1) ppm on dry matter basis. 2) Range of dietary Mo level. 3) Mean±S.D.

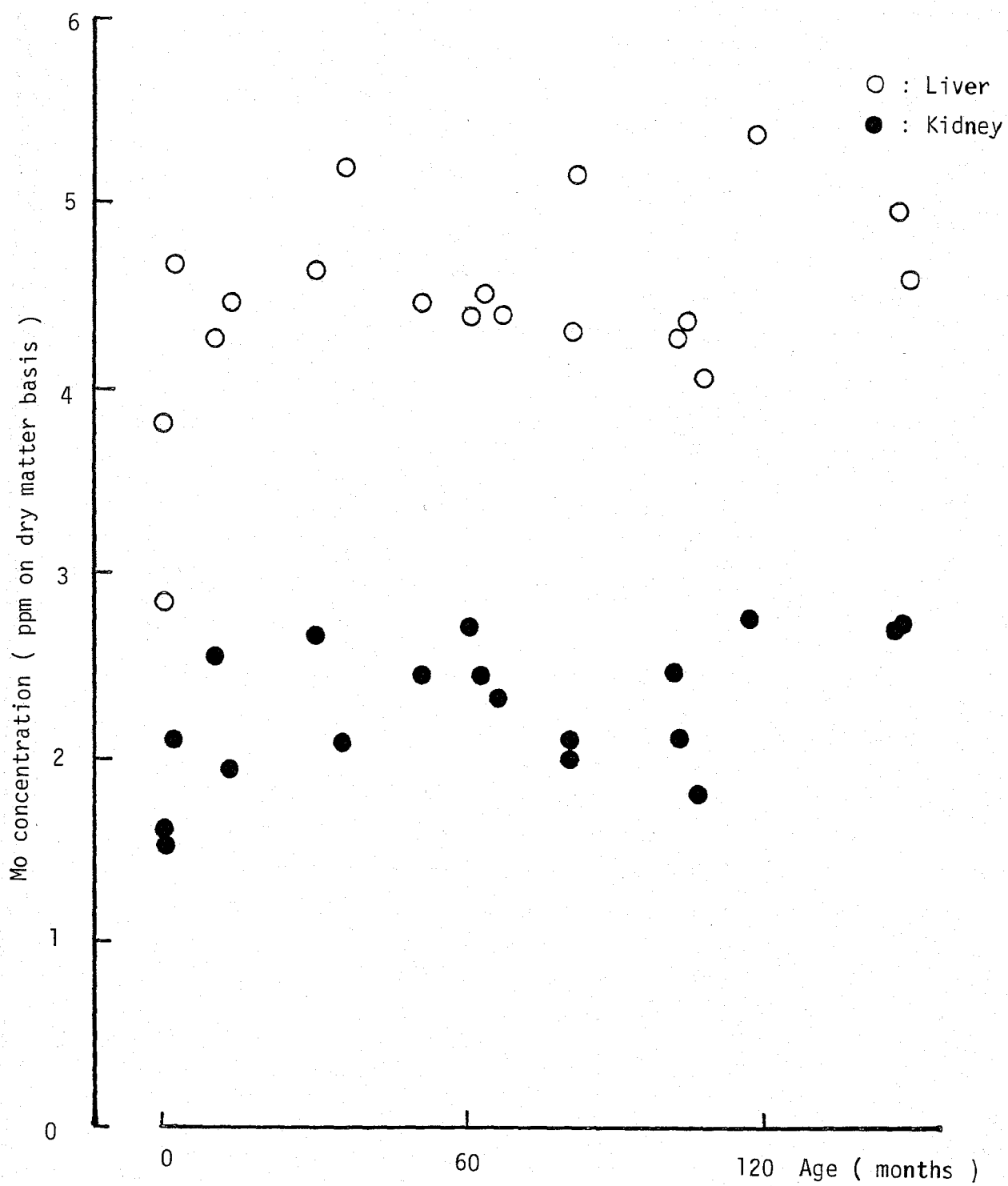


Fig. 6. Molybdenum concentration in liver and kidney of Holstein cattle kept at Kyushu Natl. Agric. Exp. Stn.



Table 28. Mo concentration in samples of Holstein cows and feed obtained from 10 dairy farms in Kumamoto prefecture

Sample	Number of samples	Mo concentration <sup>1)</sup>	
		Mean±S.D.	Range
Cow			
Liver	16	4.52±0.40	3.87-4.94
Kidney	18	2.30±0.22	1.99-2.70
Feces	18	1.45±0.28	1.07-2.08
Feed			
Concentrate <sup>2)</sup>	7	0.89±0.13	0.63-1.03
Corn ( soiling and silage )	6	0.64±0.26	0.31-1.02
Italian ryegrass ( hay )	1	1.14	
Mixed grass ( hay )	1	1.19	
Wild grass ( hay )	1	0.75	
Rice straw	4	0.68±0.26	0.46-1.06
Citrus pulp	4	0.30±0.16	0.18-0.53
Tofu pulp	1	1.16	
Whisky by-product	1	0.66	

1) ppm on dry matter basis. 2) Commercial formula feed

Table 29. Molybdenum concentration in feces, urine, milk, blood, and hair of 2 molybdenum-treated cows<sup>1)</sup>

Item	Number of collections <sup>4)</sup>	(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O administration		
		Pre-treatment <sup>5)</sup>	Treatment period <sup>5)</sup>	Post-treatment <sup>5)</sup>
Feces <sup>2)</sup>	9	1.12±0.16 <sup>6)</sup>	15.4±0.9 <sup>6)</sup>	1.19±0.12 <sup>7)</sup>
Urine <sup>3)</sup>	3	0.02±0.01	0.34±0.13	0.03±0.00
Milk <sup>2)</sup>	3	0.39±0.05	0.56±0.13	0.36±0.04
Blood <sup>2)</sup>	2	0.18±0.04	0.70±0.23	0.17±0.04
Hair <sup>2)</sup>	1	0.69±0.24	2.16±0.62	1.21

1) administered 0.1 g of Mo as (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O daily for 4 weeks. 2) ppm on dry matter basis. 3) ppm on wet matter basis. 4) obtained from a cow. 5) Each period consists of 4 weeks. 6) Mean±S.D. of collections of 2 cows. 7) Mean±S.D. of collections of a cow.

Table 30. Molybdenum concentration in tissues of molybdenum-treated and untreated cattle

Group	untreated <sup>1)</sup>					treated	
	1	2	3	4	5	15 <sup>2)</sup>	16 <sup>3)</sup>
Cattle No.							
Age ( mos. )	newborn	9	65	79	115	29	42
Body weight ( kg )	54	205	574	500	690	446	512
Liver	3.82 <sup>4)</sup>	4.24	4.42	4.32	5.40	3.99	6.90
Kidney	1.61	2.55	2.34	2.02	2.75	2.57	2.11
Heart	0.15	0.27	0.09	0.20	0.30	0.88	0.73
Lung	0.55	0.27	0.95	1.26	1.27	0.68	1.24
Pancreas	---	0.38	0.61	0.63	0.37	1.02	1.19
Spleen	0.25	1.56	7.85	---	12.98	7.27	17.58
Gall bladder	---	0.11	---	---	0.38	0.18	0.73
Diaphragm	---	0.16	0.14	0.23	0.14	0.25	0.79
M. Biceps femoris	0.15	0.15	0.15	0.12	0.33	0.15	0.54
M. Longissimus thoracis	---	0.25	0.14	0.17	0.22	0.49	0.43
Rumen	---	0.16	0.22	0.32	0.57	0.15	0.88
Reticulum	---	0.16	---	---	0.54	0.63	0.75
Omasum	---	0.26	---	---	0.42	0.40	1.86
Abomasum	0.15	0.12	---	---	0.33	0.19	0.70
Small intestine	0.08	0.35	0.20	0.38	0.33	0.15	0.87
Large intestine	0.12	0.23	0.10	0.05	0.34	0.15	0.79
Uterus	---	0.14	---	---	0.31	0.15	0.65
Urinary bladder	---	0.09	---	---	0.13	0.31	0.52
Skin	---	0.13	---	---	0.44	0.13	1.12

1) 5 cattle of different ages from the 19 cattle shown in Fig. 6.

2) slaughtered 4 weeks after the termination of the administration.

3) slaughtered immediately after the administration. 4) ppm on dry matter basis.

## Section 7. Effect of dietary Co level on Co nutrition of cattle

The essentiality of Co in ruminant nutrition reflects the importance of its role as an essential component of vitamin B<sub>12</sub> ( 142, 230 ). As Co intake declines, the synthesis of vitamin B<sub>12</sub> by rumen microorganism is inhibited and tissue concentrations of vitamin B<sub>12</sub> fall as reserves are consumed ( 142, 230 ).

Cobalt is of tremendous practical importance in ruminant nutrition, and Co deficiency was widespread in grazing ruminants on a wide range of soils and climates in areas from the tropics to cool temperate regions ( 3, 142, 149, 230 ). Cobalt deficiency in ruminants has been reported in Japan, and there is an old local disease of Co deficiency in the cattle which has been called " Kuwazu disease " in the district of Komatsu, Shiga prefecture ( 43, 218 ). Since Co content in Japanese pasture ( 99-104 ) is almost below 0.1 ppm of dietary Co requirement for dairy cattle ( NRC ( 149 )), Co deficiency appears to be the major problem in the practical feeding of dairy cattle. The objective of this section was, therefore, to clarify the effect of dietary Co level on the Co status of Holstein cattle.

### Materials and Methods

#### 1. Investigation of Co concentration in feed and tissues of cattle

The experiment was conducted as previously described ( Section 1. ).

#### 2. Effect of high Co level in rations on Co status in the body

Two lactating cows were fed 0.05 g of Co as cobalt sulfate mixed in their concentrate, daily for 4 weeks. One cow was slaughtered immediately after the administration, while the other one was slaughtered 4 weeks after the termination of the administration. The experiment was performed from 4 weeks before Co administration to slaughter and consisted of 3

periods: pre-treatment, treatment, and post-treatment. Both cows were fed 5 kg concentrate, 2 kg beet pulp, and Italian ryegrass hay ad libitum. They received water and mineral salt as a block ad libitum. They were slaughtered at 45 and 53 months old, and 514 and 582 kg in body weight, respectively.

Feces were collected 9 times each period with grab sampling from the rectum, and urine and milk were collected 3 times each period. Blood samples were obtained 2 times each period by jugular puncture. Hair was collected just at the termination of each period from the portion around the scapula. Feed samples were taken randomly. Tissues of treated cows were obtained immediately after slaughter. Five untreated cattle were chosen on the basis of age, and their tissues were obtained as described above.

### 3. Co analysis of samples

Preparation of samples was conducted as previously described ( Section 2. ). Samples were digested in nitric-perchloric acid and Co concentration was determined by graphite furnace atomic absorption spectrophotometry after extraction with chloroform ( 119 ). Each value, except for urine, is expressed on a dry matter basis. Statistical differences were evaluated by students t'test.

## Results

### 1. Investigation of Co concentration in feed and tissues of cattle

Cobalt concentration in feed at the station is shown in Table 31. Cobalt concentration in beet pulp and Tall oatgrass was 2-4 fold lower than that in concentrate and Italian ryegrass. Cobalt concentration in water was below 0.01 ppm. From the figures for Co concentration in feed and feed intake, it was assumed that Co levels in total diets of dry, lactating, and fattening cows were 0.14-0.16, 0.14-0.16, and 0.12-0.15

ppm, respectively.

Cobalt concentration in the liver, kidney, blood, and feces of cows at the station is shown in Table 32. There were no significant differences between groups in Co concentration in the liver, kidney, blood, and feces, but that in the feces of lactating and fattening cows was slightly higher than for dry cows.

Cobalt concentration in the liver and kidney of 19 cattle kept at the station is shown in Fig. 7. Cobalt concentrations in the liver and kidney were 0.05–0.07 and 0.02–0.04 ppm respectively in 2 newborn and one 1 month old calves, but those in 2 heifers were 0.12–0.20 and 0.05 ppm, respectively. Also, those in 14 cows were 0.09–0.32 and 0.05–0.14 ppm, respectively. Cobalt concentration in the liver and kidney of calves was lower than for cows. Also, Co concentration in the liver was higher than in the kidney, and that in the liver and kidney of cows was not affected by age.

Cobalt concentration in samples of cows and feed obtained from 10 dairy farms is shown in Table 33. Cobalt concentration in concentrate, Italian ryegrass, rice straw, citrus pulp, and tofu pulp was below 0.1 ppm. Cobalt concentration in the liver, kidney, and feces was almost similar to that at the station.

## 2. Effect of high Co level in rations on Co status in the body

Feed intake of 2 cows was not decreased by Co administration. Daily milk yields of treated cows were 10–15 and 14–18 kg, respectively, and not affected by Co administration. Cobalt concentrations in concentrate, beet pulp, and Italian ryegrass hay were 0.21, 0.14, and 0.18 ppm, respectively. It was assumed that daily Co intake and dietary Co level in the pre-treatment and post-treatment periods were 2.7 mg and 0.19 ppm, respectively, whereas those in the treatment period were 53 mg and 3.4 ppm, respectively.

Cobalt concentration in the feces, urine, milk, blood, and hair of 2

treated cows is shown in Table 34. Cobalt concentration in the feces in the treatment period was about 18 fold higher than before or after treatment, and that in the urine was about 10-20 fold higher. However, Co concentration in the milk, blood, and hair in the treatment period was almost similar to before or after treatment. Assuming that digestibility of dry matter and daily urine amounts were 65 % and 10 kg, respectively, daily Co excretion in the feces, urine, and milk of the pre-treatment and post-treatment periods were 3.7, 0.02, 0.02 mg, respectively, whereas those of the treatment period were 65, 0.2, and 0.02 mg, respectively.

Cobalt concentration in the tissues of treated and untreated cattle is shown in Table 35. Higher Co concentration in the untreated cattle was obtained from the liver and kidney. Cobalt concentration in most tissues of the cow slaughtered 4 weeks after the termination of the administration was almost similar to that for untreated cattle. Also, Co concentration in most tissues of the other cow slaughtered immediately after the administration was higher than for untreated cattle; that in the rumen, reticulum, omasum, liver, and kidney was very high, and that in the rumen content was 10.0 ppm.

## Discussion

The minimum Co requirement of dairy cattle is about 0.1 ppm ( NRC ( 149 ) ). Although some Co concentration in feed was below 0.1 ppm, Co intake by the cattle was almost sufficient in this study. Furthermore, assuming that daily intake of mineral salt as a block was about 10-20 g in this study, daily Co intake from mineral salt was 1-2 mg. Therefore, Co intake by the cattle may be sufficient, if mineral salt as a block was given ad libitum. However, there may be the possibility of a borderline Co deficiency for the cattle fed rations of low Co content in Japan, since Co

content of feed was usually below 0.1 ppm in this study and earlier papers ( 99-104 ).

Levels in the liver have attracted special attention because of their possible value in diagnosing Co deficiency of ruminants in the field ( 230 ). Also, in two Australian studies the mean liver Co levels of groups of Co deficient sheep were 0.06 and 0.09 ppm ( d.b. ), and other worker suggests that 0.04-0.06 ppm Co or less in the livers of sheep and cattle indicate Co deficiency and 0.08-0.12 ppm Co indicate a satisfactory Co status ( 230 ). Liver Co concentration in this study was regarded as normal, since that was above 0.09 ppm. However, it is apparent that freedom from signs of Co deficiency is compatible with a relatively low liver Co level and a deficiency can occur in the presence of a high liver Co level ( 230 ). For these reasons liver Co concentration, although of some practical value, is not an entirely reliable criterion of the Co-vitamin B<sub>12</sub> status of ruminants, and liver vitamin B<sub>12</sub> provides a reliable biochemical assessment of the Co status ( 230 ). Therefore, more attention must be paid to Co deficiency in feeding dairy cattle, although Co intake was almost sufficient in this study.

According to NRC standards ( 149 ), 10 ppm Co in the dry diet is usually accepted as a safe level. Cobalt toxicity of the cattle may not occur in Japan, since Co concentration in feed in this study and earlier papers ( 99-104 ) was below 1 ppm. Also, excessive accumulation does not occur in any particular organ or tissues, but the liver, kidney, and bones usually carry the highest concentration of Co ( 142, 230 ). In this study, higher Co concentration of Co-treated cows was obtained from the rumen, reticulum, omasum, liver, and kidney. Furthermore, Co concentration in the feces and urine of the treatment period was higher than before or after treatment, but not in the milk, blood, and hair. It was, therefore, suggested that most of Co administered was excreted in the feces and



urine, but some Co accumulated in the body.

The Co content in the liver of the newborn lamb and calf is reduced below normal when the mother has been on a Co-deficient diet, and that can be raised to normal levels by prepartum Co administration ( 230 ). Also, it is indicated that Co does not normally accumulated in the fetal liver ( 230 ). Although liver Co concentration of newborn calves born from cows fed sufficient Co in this study was lower than for cows, it is not apparent whether there was borderline Co deficiency fo the calves in this study. Further study may, therefore, be needed on Co nutrition of cattle, since Co deficiency in the practical feeding of the cattle has become recognized as a major problem in Japan.

Table 31. Cobalt concentration in feed obtained from  
Kyushu Natl. Agric. Exp. Stn.

Feed	Number of samples	Co concentration <sup>1)</sup>
Concentrate	4	0.16±0.06 <sup>2)</sup>
Beet pulp	2	0.08±0.02
Italian ryegrass ( hay )	2	0.13±0.07
Italian ryegrass ( low moisture silage )	2	0.16±0.08
Tall oatgrass ( hay )	2	0.04±0.01

1) ppm on dry matter basis. 2) Mean±S.D.

Table 32. Cobalt concentration in liver, kidney, blood and feces of Holstein cows kept at Kyushu Natl. Agric. Exp. Stn.

	Dry	Lactating	Fattening
Number of cows	6	4	4
Dietary Co level <sup>1)</sup>	0.14-0.16 <sup>2)</sup>	0.14-0.16	0.12-0.15
Liver <sup>1)a)</sup>	0.15±0.08 <sup>3)</sup>	0.20±0.08	0.12±0.02
Kidney <sup>1)a)</sup>	0.08±0.04	0.08±0.03	0.07±0.01
Blood <sup>1)a)</sup>	0.014±0.001	0.014±0.003	0.015±0.003
Feces <sup>1)a)</sup>	0.35±0.20	0.47±0.20	0.51±0.09

a: Values in the same line are not significantly different (  $P>0.05$  )

1) ppm on dry matter basis. 2) Range of dietary Co level. 3) Mean±S.D.

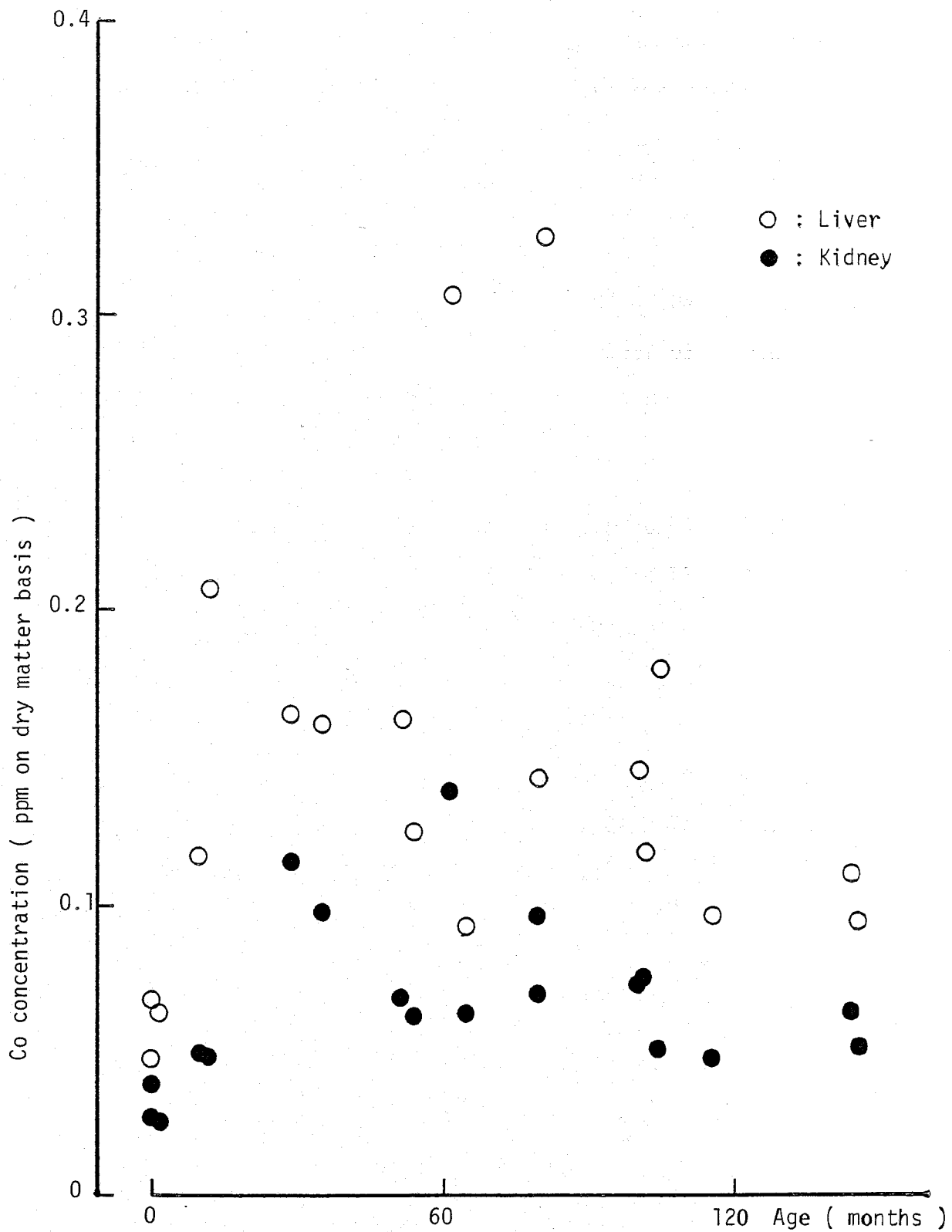


Fig. 7. Cobalt concentration in liver and kidney of Holstein cattle kept at Kyushu Natl. Agric. Exp. Stn.

Table 33. Cobalt concentration in samples of Holstein cows and feed obtained from 10 dairy farms in Kumamoto prefecture

Sample	Number of samples	Co concentration <sup>1)</sup>	
		Mean±S.D.	Range
Cow			
Liver	16	0.16±0.04	0.09-0.25
Kidney	18	0.06±0.02	0.03-0.11
Feces	18	0.31±0.12	0.19-0.57
Feed			
Concentrate <sup>2)</sup>	7	0.07±0.03	0.05-0.14
Corn	6	0.16±0.12	0.03-0.32
( soiling and silage )			
Italian ryegrass	1	0.06	
( hay )			
Mixed grass	1	0.21	
( hay )			
Wild grass	1	0.13	
( hay )			
Rice straw	4	0.09±0.02	0.07-0.11
Citrus pulp	4	0.04±0.02	0.03-0.06
Tofu pulp	1	0.03	
Whisky by-product	1	0.13	

1) ppm on dry matter basis. 2) Commercial formula feed

Table 34. Cobalt concentration in feces, urine, milk, blood, and hair of 2 cobalt-treated cows<sup>1)</sup>

Item	Number of collections <sup>4)</sup>	CoSO <sub>4</sub> ·7H <sub>2</sub> O administration		
		Pre-treatment <sup>5)</sup>	Treatment period <sup>5)</sup>	Post-treatment <sup>5)</sup>
Feces <sup>2)</sup>	9	0.74±0.08 <sup>6)</sup>	13.0±1.6 <sup>6)</sup>	0.72±0.06 <sup>7)</sup>
Urine <sup>3)</sup>	3	0.001±0.000	0.020±0.011	0.002±0.001
Milk <sup>2)</sup>	3	0.010±0.005	0.012±0.002	0.010±0.005
Blood <sup>2)</sup>	2	0.007±0.004	0.010±0.002	0.008±0.003
Hair <sup>2)</sup>	1	0.036±0.030	0.036±0.006	0.026

1) administered 0.05 g of Co as CoSO<sub>4</sub>·7H<sub>2</sub>O daily for 4 weeks. 2) ppm on dry matter basis. 3) ppm on wet matter basis. 4) obtained from a cow. 5) Each period consists of 4 weeks. 6) Mean±S.D. of collections of 2 cows. 7) Mean±S.D. of collections of a cow.

Table 35. Cobalt concentration in tissues of cobalt-treated and untreated cattle

Group	untreated <sup>1)</sup>					treated	
	1	2	3	4	5	17 <sup>2)</sup>	18 <sup>3)</sup>
Cattle No.							
Age ( mos. )	newborn	9	65	79	115	45	53
Body weight ( kg )	54	205	574	500	690	514	592
Liver	0.07 <sup>4)</sup>	0.12	0.09	0.32	0.10	0.15	0.55
Kidney	0.04	0.05	0.06	0.09	0.05	0.08	0.16
Heart	0.02	0.02	0.02	0.03	0.01	0.04	0.06
Lung	0.01	0.05	0.02	0.03	0.01	0.05	0.03
Pancreas	---	0.03	0.04	0.04	0.03	0.04	0.06
Spleen	0.01	0.02	0.03	---	0.01	0.05	0.03
Gall bladder	---	0.03	---	---	0.01	0.01	0.02
Diaphragm	---	0.02	0.04	0.02	0.01	0.01	0.02
M. Biceps femoris	0.02	0.02	0.02	0.02	0.01	0.01	0.02
M. Longissimus thoracis	---	0.02	0.02	0.01	0.02	0.01	0.02
Rumen	---	0.04	0.02	0.02	0.02	0.04	1.20
Reticulum	---	0.03	---	---	0.04	0.04	0.95
Omasum	---	0.03	---	---	0.02	0.08	1.08
Abomasum	0.02	0.02	---	---	0.01	0.03	0.04
Small intestine	0.02	0.03	0.02	0.02	0.04	0.02	0.05
Large intestine	0.02	0.03	0.04	0.02	0.02	0.01	0.08
Uterus	---	0.01	---	---	0.01	0.01	0.04
Urinary bladder	---	0.02	---	---	0.01	---	0.02
Skin	---	0.04	---	---	0.03	0.02	0.06

1) 5 cattle of different ages from the 19 cattle shown in Fig. 7.

2) slaughtered 4 weeks after the termination of the administration.

3) slaughtered immediately after the administration. 4) ppm on dry matter basis.

## Section 8. Effect of dietary Cd level on Cd nutrition of cattle

Recently, Cd is shown to be probably essential for animals, although it has been recognized as a toxic element for a long time ( 230 ). Also, clinical symptoms of Cd toxicity in cattle has not been reported under practical conditions, although the Cd toxicity in human, " Itai-itai disease ", had been reported in Japan. With current dairy cattle feeding and management conditions, Cd toxicity is relatively unimportant, because most feed and forages contain little Cd and only a very small amount of ingested Cd is absorbed ( 142, 230 ). However, there may be the possibility of a borderline Cd toxicity for the cattle, since Cd contamination of soil and rice has been reported in many areas in Japan ( 160 ).

The objective of this section was, therefore, to clarify the effect of dietary Cd level on the Cd status of Holstein cattle.

### Materials and Methods

#### 1. Investigation of Cd concentration in feed and tissues of cattle

The experiment was conducted as previously described ( Section 1. ).

#### 2. Effect of high Cd level in rations on Cd status in the body

Two lactating cows were fed 1.25 g of Cd as cadmium chloride mixed in their concentrate, daily for 4 weeks. One cow was slaughtered immediately after the administration, while the other one was slaughtered 4 weeks after the termination of the administration. The experiment was performed from 4 weeks before Cd administration to slaughter and consisted of 3 periods: pre-treatment, treatment, and post-treatment. Both cows were fed 4-5 kg concentrate, 1-2 kg beet pulp, and Italian ryegrass hay and low moisture silage ad libitum. They received water and mineral salt as a



block ad libitum. They were slaughtered at 112 and 112 months old, and 664 and 656 kg in body weight, respectively.

Feces were collected 2 times each period with grab sampling from the rectum, and urine and milk were collected 2 times each period. Blood and hair were collected just at the termination of each period; blood was obtained by jugular puncture, and hair was collected from the portion around the scapula. Feed samples were taken randomly. Tissues of treated cows were obtained immediately after slaughter. Five untreated cattle were chosen on the basis of age, and their tissues were obtained as described above.

### 3. Cd analysis of samples

Preparation of samples was conducted as previously described ( Section 2. ). Samples were digested in nitric-perchloric acid and Cd concentration was determined by atomic absorption spectrophotometry after extraction with methyl isobutyl ketone ( 217 ). Each value, except for urine, is expressed on a dry matter basis. Statistical differences were evaluated by students t'test.

## Results

### 1. Investigation of Cd concentration in feed and tissues of cattle

Cadmium concentration in the liver and kidney of 19 cattle kept at the station is shown in Fig. 8. Cadmium concentration in the liver and kidney were 0.05–0.14 and 0.06–0.10 ppm respectively in 2 newborn and one 1 month old calves, but those in 3 cows of 115–145 months old were 1.33–1.90 and 8.98–13.39 ppm, respectively. Cadmium concentration in the liver and kidney increased with aging, and those in 3 cows of 115–145 months old were 14–18 and 112–152 fold higher than for calves. Also, the regression equation of Cd concentration of liver (  $Y_{l_i}$  ) and kidney (  $Y_{k_i}$  ) on age

( X ) were as follows:

$$Yl_1 = 0.11^{**}X + 0.14 \quad ( ** : P < 0.01 )$$

$$Yk_1 = 0.91^{**}X - 0.22 \quad ( ** : P < 0.01 )$$

The weight of the liver and kidney of 14 cattle kept at the station is shown in Fig. 9. The weights of liver and kidney were 1.40 and 0.41 kg respectively in one month old calf, but those in 144 months old cow were 8.40 and 1.57 kg, respectively. The weights of liver and kidney increased with aging, and those in 144 months old were 6 and 4 fold higher than for 1 month old calf. Also, the regression equation of the weight of the liver (  $Yl_2$  ) and kidney (  $Yk_2$  ) on age ( X ) were as follows:

$$Yl_2 = 0.59^{**} X + 2.78 \quad ( ** : P < 0.01 )$$

$$Yk_2 = 0.12^{**} X + 0.56^{**} \quad ( ** : P < 0.01 )$$

The accumulated Cd content in the liver and kidney of 14 cattle kept at the station is shown in Fig. 10. from the figures for Fig. 8., Fig. 9., and the mean water content ( Liver: 71.5, Kidney: 75.7 % ( 173 ) ). The accumulated Cd contents in the liver and kidney were 0.03 and 0.01 mg respectively in one 1 month old calf, but those in one 144 months old cow were 3.18 and 3.42 mg, respectively. The accumulated Cd contents of the liver and kidney increased with aging, and those in 144 months old cow were 106 and 342 fold higher than for 1 month old calf. Also, the regression equations of the accumulated Cd of the liver (  $Yl_3$  ) and kidney (  $Yk_3$  ) on age ( X ) were as follows:

$$Yl_3 = 0.30^{**}X - 0.18 \quad ( ** : P < 0.01 )$$

$$Yk_3 = 0.31^{**}X - 0.27 \quad ( ** : P < 0.01 )$$

Cadmium concentration in feed at the station is shown in Table 36. Cadmium concentration in Italian ryegrass and Tall oatgrass was 2-3 fold lower than that in concentrate and beet pulp. Cadmium concentration in water was below 0.01 ppm. From the figures for Cd concentration in feed and feed intake, it was assumed that Cd levels in total diets of dry,

lactating, and fattening cows were 0.13–0.20 ppm.

Cd concentration in samples of cows and feed obtained from 10 dairy farms is shown in Table 37. Cadmium concentration in feed varied widely, and that in the rice straw was considerably higher. Cadmium concentration in the liver and kidney on dairy farms was almost similar to that at the station.

## 2. Effect of high Cd level in rations on Cd status in the body

Feed intake of 2 cows was not decreased by Cd administration. Daily milk yields of treated cows were 8–12 and 12–14 kg, respectively, and not affected by Cd administration. Cadmium concentration in concentrate, beet pulp, Italian ryegrass hay and low moisture silage were 0.10, 0.27, 0.13, and 0.11 ppm, respectively. It was assumed that daily Cd intake and dietary Cd level in the pre-treatment and post-treatment periods were 2 mg and 0.13 ppm, respectively, whereas those in the treatment period were 1.25 g and 83 ppm, respectively.

Cadmium concentration in the feces, urine, milk, blood, and hair of 2 treated cows is shown in Table 38. Cadmium concentration in the feces in the treatment period was about 200–300 fold higher than before or after treatment, and that in the blood and hair was 5–14 fold higher. However, Cd concentration in the urine and milk was not detected in each period. Assuming that digestibility of dry matter was 65 %, daily Cd excretion in the feces of the pre-treatment and post-treatment periods was 3.8–6.0 mg, whereas that in the treatment period was 1.15 g.

Cadmium concentration in the tissues of treated and untreated cattle is shown in Table 39. Higher Cd concentration in the untreated cattle was obtained from the kidney and liver. Cadmium concentration in most tissues of the cow slaughtered 4 weeks after the termination of the administration was almost similar to that for untreated cattle, but that in the kidney, liver, and pancreas was considerably higher. Also, Cd concentration in the

tissues of the other cow slaughtered immediately after the administration was higher than for untreated cattle, but that in the kidney and liver was almost similar to that of the cow slaughtered 4 weeks after the termination of the administration.

## Discussion

Although Cd is highly toxic to dairy cattle, clinical symptoms of dairy cattle have not been reported under practical conditions ( 142, 230 ). A reduction in growth, feed intake, and water consumption occurred in calves fed 160 ppm Cd, but there was no significant effect with 40 ppm ( 181 ). Likewise, milk production was drastically decreased in lactating cows given 3 g of Cd as  $\text{CdCl}_2$  per day ( 132 ). However, feed intake and milk production was not affected by the administration of 83 ppm Cd in the diet in this study. Furthermore, most feed contained less than 1 ppm Cd in this study. Therefore, Cd toxicity may be not occurred with current dairy cattle feeding and management conditions, because most feed and forage in Japan contain little Cd except for Cd contamination of soil and feed. However, a borderline toxicity appears possible where animals are fed certain types of recycled waste such as sewage sludge in which Cd is concentrated ( 230 ). Thus, it may be necessary to pay attention to Cd concentration in feed, because Cd contamination of soil and rice has been reported in Japan ( 160 ) and Cd concentration in rice straw was considerably higher.

Cattle had much more effective mechanisms for keeping Cd out of the body tissues than for excreting it once it is absorbed ( 28, 142, 157, 181 ). In one study ( 136 ), lactating cows retained only 0.75 % of radioactive Cd given orally, and in a comparable study ( 156 ), young goats absorbed and retained only 0.34 %. In this study Cd concentration in

the feces of the treatment period was higher than before or after treatment, and higher Cd concentration of Cd-treated cows was obtained from most tissues. It was suggested that most of Cd administered was excreted in the feces, but some Cd accumulated in the body.

Cadmium is virtually absent from the human body at birth and accumulate with age up to about 50 years ( 160 ). Also, much of the retained Cd is in the liver and kidney, which together contain as high as 75 % of the total body content ( 142 ). In this study, Cd concentration in the liver and kidney increased with aging, but not in the other tissues. Also, retained Cd was particularly accumulated in the liver and kidney by the administration of 83 ppm Cd. It is, therefore, suggested that cattle retained Cd in the liver and kidney for a long time, once absorbed. However, very little Cd is found in muscle and milk than that in the other food ( 69, 70, 83, 108, 147, 148, 209-212 ). Due to low absorption and an effective screening mechanism, dairy cattle are very effective in keeping Cd levels in milk and muscle meat very low ( 142, 230 ). Thus, dairy cattle protect the consumer from the adverse effect of Cd. Furthermore, relatively soon after absorption, Cd combines with protein, perhaps which greatly reduces its toxicity ( 142, 230 ). However, It may be necessary to pay attention to the interaction between Cd and other elements, since some of the toxic effects of Cd can be diminished or prevented by Zn, Co, Se, and thio compounds ( 142, 230 ).

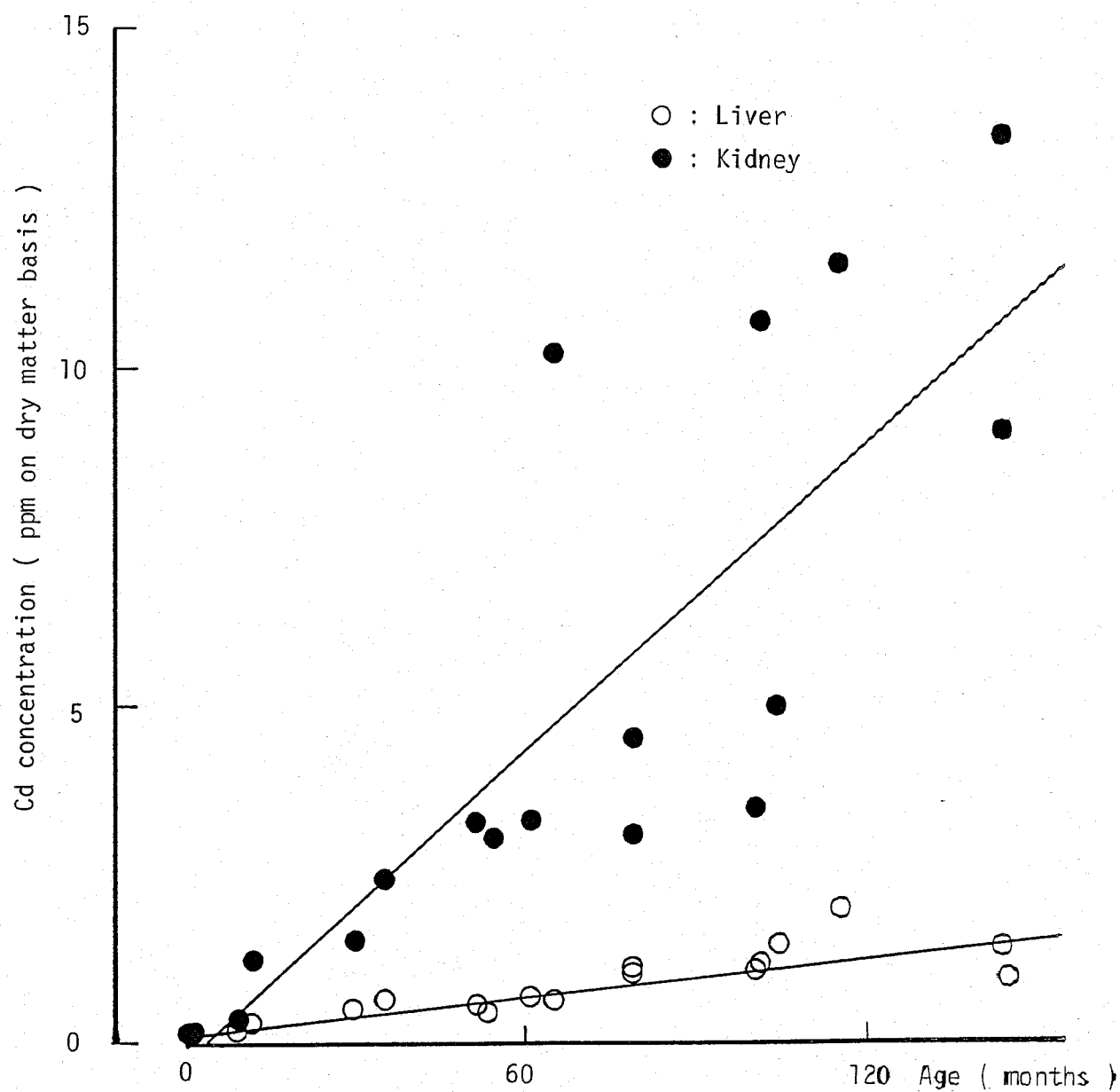


Fig. 8. Cadmium concentration in liver and kidney of **Holstein** cattle kept at Kyushu Natl. Agric. Exp. Stn.

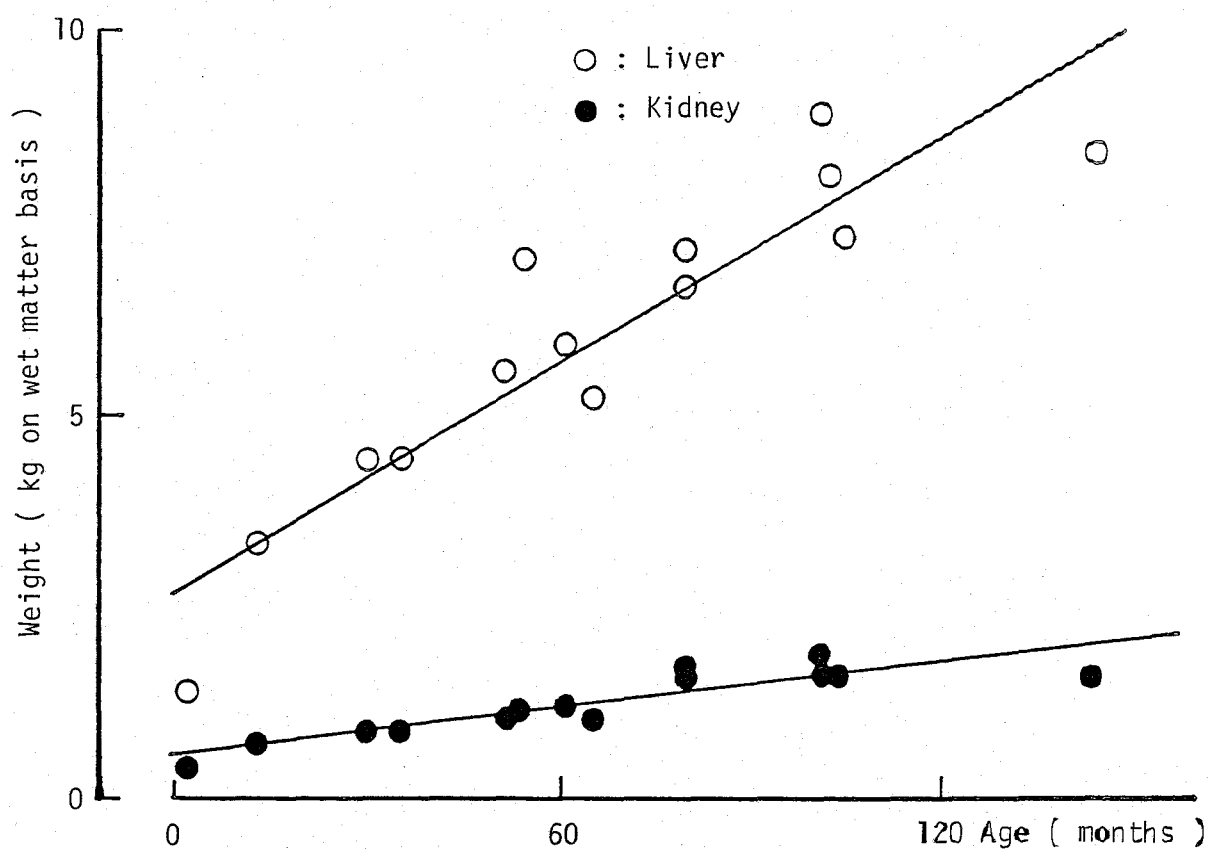


Fig. 9. Weight of liver and kidney of Holstein cattle kept at Kyushu Natl. Agric. Exp. Stn.

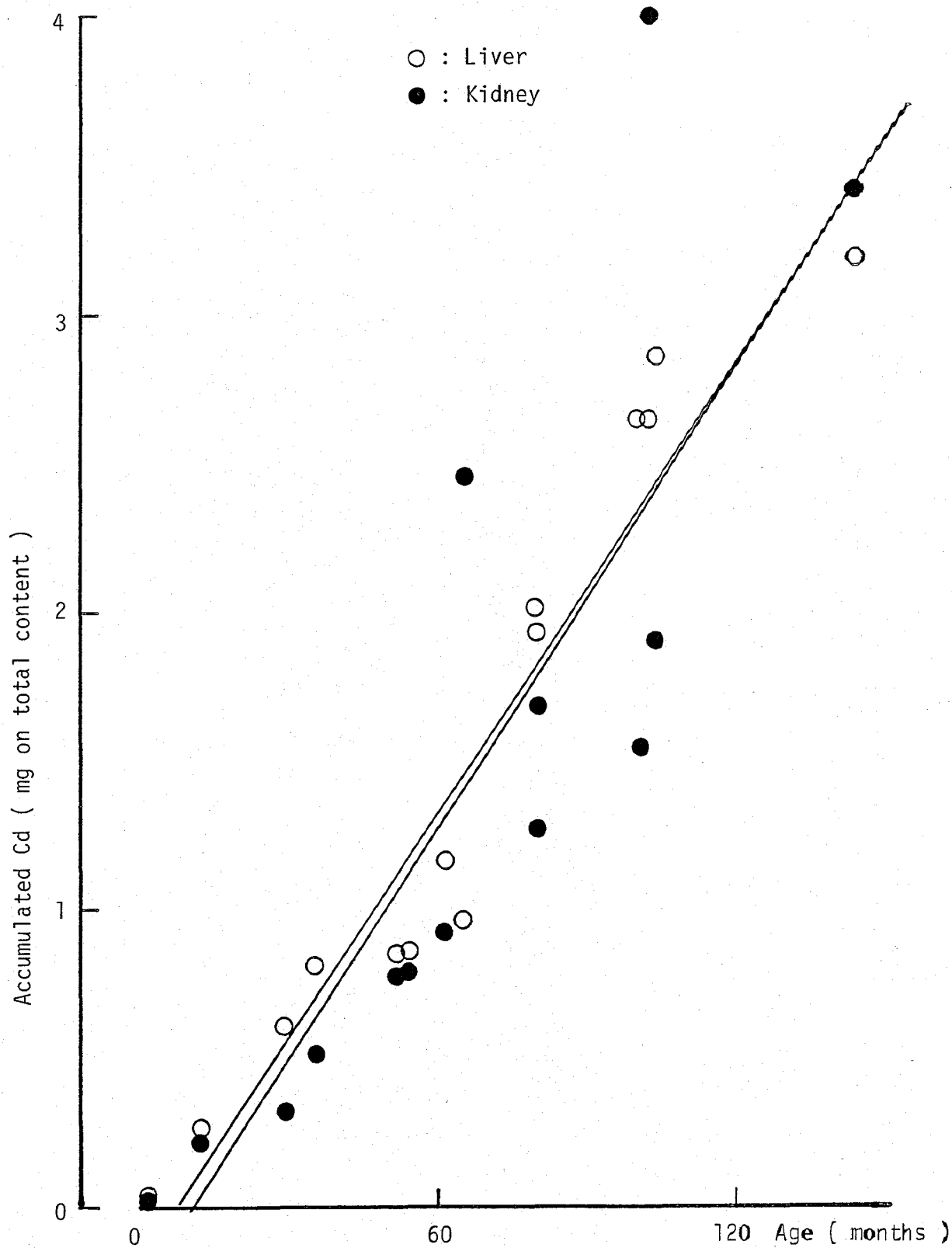


Fig. 10. Accumulated cadmium in liver and kidney of Holstein cattle kept at Kyushu Natl. Agric. Exp. Stn.



Table 36. Cadmium concentration in feed obtained from  
Kyushu Natl. Agric. Exp. Stn.

Feed	Number of samples	Cd concentration <sup>1)</sup>
Concentrate	4	0.23±0.06 <sup>2)</sup>
Beet pulp	2	0.28±0.14
Italian ryegrass ( hay )	2	0.13±0.03
Italian ryegrass ( low moisture silage )	2	0.12±0.04
Tall oatgrass ( hay )	2	0.08±0.01

1) ppm on dry matter basis. 2) Mean±S.D.

Table 37. Cadmium concentration in samples of Holstein cows and feed obtained from 10 dairy farms in Kumamoto prefecture

Sample	Number of samples	Cd concentration <sup>1)</sup>	
		Mean±S.D.	Range
Cow			
Liver	16	0.71±0.18	0.44-1.05
Kidney	18	3.65±1.30	1.60-5.60
Feces	18	0.41±0.22	0.13-0.97
Feed			
Concentrate <sup>2)</sup>	7	0.22±0.04	0.19-0.29
Corn ( soiling and silage )	6	0.33±0.22	0.08-0.67
Italian ryegrass ( hay )	1	0.22	
Mixed grass ( hay )	1	0.18	
Wild grass ( hay )	1	0.28	
Rice straw	4	0.58±0.58	0.17-1.43
Citrus pulp	4	0.01±0.01	ND-0.02
Tofu pulp	1	0.04	
Whisky by-product	1	0.19	

ND: below 0.01 ppm. 1) ppm on dry matter basis. 2) Commercial formula feed

Table 38. Cadmium concentration in feces, urine, milk, blood, and hair of 2 cadmium-treated cows<sup>1)</sup>

Item	Number of collections <sup>4)</sup>	CdCl <sub>2</sub> ·2.5H <sub>2</sub> O administration		
		Pre-treatment <sup>5)</sup>	Treatment period <sup>5)</sup>	Post-treatment <sup>5)</sup>
Feces <sup>2)</sup>	2	0.72±0.07 <sup>6)</sup>	218.7±26.3 <sup>6)</sup>	1.16±0.06 <sup>7)</sup>
Urine <sup>3)</sup>	2	ND	ND	ND
Milk <sup>2)</sup>	2	ND	ND	ND
Blood <sup>2)</sup>	1	ND	0.14±0.01	ND
Hair <sup>2)</sup>	1	0.18±0.01	1.68±0.85	0.33

1) administered 1.25 g of Cd as CdCl<sub>2</sub>·2.5H<sub>2</sub>O daily for 4 weeks. 2) ppm on dry matter basis. 3) ppm on wet matter basis. 4) obtained from a cow. 5) Each period consists of 4 weeks. 6) Mean±S.D. of collections of 2 cows. 7) Mean±S.D. of collections of a cow. ND: below 0.01 ppm.

Table 39. Cadmium concentration in tissues of cadmium-treated and untreated cattle

Group	untreated <sup>1)</sup>					treated	
	1	2	4	5	6	19 <sup>2)</sup>	20 <sup>3)</sup>
Cattle No.							
Age ( mos. )	newborn	9	79	115	145	112	112
Body weight ( kg )	54	205	500	690	696	664	656
Liver	0.05 <sup>4)</sup>	0.16	0.53	1.90	0.93	13.82	15.33
Kidney	0.06	0.25	3.04	11.48	13.39	54.22	53.17
Heart	0.05	0.05	0.11	0.10	0.09	0.03	0.23
Lung	0.14	0.20	0.19	0.21	0.07	0.30	0.74
Pancreas	---	0.03	0.10	0.06	---	1.97	1.87
Spleen	0.05	0.06	---	0.26	0.22	0.24	0.73
Gall bladder	---	0.09	---	0.23	---	0.02	0.69
Diaphragm	---	0.03	0.06	0.03	0.03	0.08	0.05
M. Biceps femoris	0.02	0.06	0.04	0.05	0.05	---	---
M. Longissimus thoracis	---	0.05	0.03	0.06	0.02	---	---
Rumen	---	0.11	0.08	0.08	0.12	0.10	2.65
Reticulum	---	0.16	---	0.24	---	0.19	0.91
Omasum	---	0.15	---	0.18	---	0.09	2.38
Abomasum	0.16	0.06	---	0.14	---	0.20	2.48
Small intestine	0.12	0.15	0.13	0.22	0.26	0.65	1.06
Large intestine	0.05	0.14	0.10	0.19	0.14	0.48	0.78
Uterus	---	0.20	---	0.33	---	0.04	0.58
Urinary bladder	---	0.16	---	0.27	---	0.07	0.09
Skin	---	0.11	---	0.02	---	ND	0.01

1) 5 cattle of different ages from the 19 cattle shown in Fig. 8.

2) slaughtered 4 weeks after the termination of the administration.

3) slaughtered immediately after the administration. 4) ppm on dry matter basis. ND: below 0.01 ppm.

## Section 9. Trace element nutrition and interaction of dairy cattle

Nutritional abnormalities involving trace elements may arise as simple deficiencies or excesses of single elements ( 142, 230 ). However, they usually occur as deficiencies or excesses conditioned by the extent to which other mineral elements, nutrients, or organic factors are capable of modifying the ability of the animal to utilize the deficient or toxic element ( 142 ). Also, the metabolism, amounts required, and maximum safe dietary levels of essential elements are affected in a major way by the level of other minerals in the diet ( 142, 230 ). Furthermore, an understanding of dietary and tissue level interrelationships between or among minerals has proved to be important in providing optimum nutrition for ruminants ( 11 ).

Recently, many of the interactions between or among minerals has been often discussed under various elements ( 3, 142, 149, 230 ). The nutrition of each trace element was investigated in the previous section ( Section 1-8. ), but trace element interactions was scarcely discussed. The objective of this section was, therefore, to clarify trace element nutrition and interaction of dairy cattle.

### Materials and Methods

#### 1. Investigation of trace element concentration in feed and tissues of cattle

The experiment was conducted as previously described ( Section 1. ).

#### 2. Effect of high trace element level in rations on trace element status in the body

The experiment was conducted as previously described ( Section 2-8. ).

#### 3. Trace element analysis of samples

Preparation of samples was conducted as previously described ( Section 2. ). Trace element analysis of samples was conducted as previously described ( Section 1-8 ). Each value is expressed on a dry matter basis.

## Results

### 1. Investigation of trace element concentration in feed and tissues of cattle

Trace element concentration in feed obtained from Kyushu Natl. Agric. Exp. Stn. and 10 dairy farms in Kumamoto prefecture is shown in Table 40, compared with NRC standards ( 149 ). Trace element concentration in feed varied widely, especially in roughage and by-product feed. Iron and Mn concentration in feed was almost above the dietary requirement of dairy cattle, but that in roughage was higher than that in concentrate. Zinc, Cu, and Se concentration in concentrate was almost above the dietary requirement, but not in roughage. Molybdenum concentration in feed was below 6 ppm dietary Mo toxicity level for dairy cattle, and Co concentration in feed was sometimes below the dietary requirement. Also, Cd concentration in feed was almost below 1 ppm.

Trace element concentration in the liver of Holstein cattle kept at Kyushu Natl. Agric. Exp. Stn. and 10 dairy farms in Kumamoto prefecture is shown in Table 41. Zinc, Cu, and Se concentration in the liver of newborn calf was higher than for cows, but Fe, Mn, Co, and Mo concentration was lower. Cadmium concentration in the liver increased with aging. Trace element concentration in the liver on dairy farms was almost similar to that at the station.

The correlation coefficient of trace element concentration in the liver of Holstein cows kept at Kyushu Natl. Agric. Exp. Stn. and 10 dairy farms in Kumamoto prefecture is shown in Table 42. Significant correlations

between trace element concentration in the liver was obtained from Fe-Se, Zn-Cu, and Cu-Se at the station, and Zn-Cu, Zn-Se, and Se-Cu on dairy farms.

## 2. Effect of high trace element level in rations on trace element status in the body

Trace element concentration in the liver of trace element-treated cows is shown in Table 43. The concentration of corresponding elements in the liver of the cow slaughtered immediately after the administration of each element was higher than for untreated cows. Also, Cu, Se, and Cd concentration in the liver of the cow slaughtered 4 weeks after the termination of the administration was higher than for untreated cows, but Zn, Mn, Mo, and Co concentration was almost similar to that for untreated cows. Except for corresponding elements by each administration, trace element concentration in the liver was almost similar to that for untreated cows, but Cu concentration in the liver by the administration of Zn was comparatively lower.

## Discussion

Trace element concentration in feed varied widely in this study. Also, Zn, Cu, and Se concentration in roughage was almost below the dietary requirement, although Fe and Mn concentration in feed was almost above the dietary requirement. Furthermore, Co concentration in feed was sometimes below the dietary requirement. Therefore, Zn, Cu, Se, and Co intake by the cattle fed rations of low those content as roughage in Japan was marginally inadequate, because those content in roughage was almost below the dietary requirement in this study and earlier reports ( 152, 203 ). Also, plant materials provide the main source of minerals to animals, and the factors influencing the trace element content in plants are therefore

major determinants of dietary intakes of cattle ( 230 ). Furthermore, trace element content of feed is extremely variable, compared to total digestible nutrients or protein ( 2 ). It is, therefore, necessary to pay attention to trace element nutrition in the practical feeding of dairy cattle, since variable feed was used in Japanese farms. Thus, sufficient trace element should be added up to the dietary requirement in the total ration. However, except for the contamination in soil and feed, trace element concentration in Japanese feed may be almost below the dietary tolerable level for dairy cattle.

All plants and animals depend ultimately on the soil for their supply of mineral nutrients ( 230 ). The wide range of trace element concentration in roughage, especially in silage and hay, may be partly due to the contamination of soil in this study, since soil contamination of roughage has been reported at harvesting roughage ( 104 ). Also, soil ingestion by the cattle, especially grazing cattle, can be beneficial to mineral nutrition ( 42, 58, 59, 104, 203, 230 ). Furthermore, most of trace element ingested was excreted in the feces and urine, and fertilized the soil as compost and barnyard manure. It was, therefore, suggested that the soil-plant-animal interrelations of trace elements should be taken into account carefully in practical feeding of dairy cattle.

Trace element requirement and tolerance carry the assumption that the whole diet is adequate and well balanced for the purpose of which it is fed, and that free from other toxic factors capable of adversely affecting the animal's health, appetite, or utilization of the element concerned ( 230 ). Also, a variety of metabolic interaction are known to exist among trace elements and affect trace element requirement in ruminants ( 61, 230 ). In this study, there were significantly positive correlations among Zn, Cu, and Se concentration in the liver. Therefore, there may be the possibility of the simultaneous occurrence of Zn, Cu, and Se deficiency



for the cattle fed mainly roughage, since the correlation among those elements in the liver was significantly positive. Thus, more attention must be paid to mineral interaction in feeding dairy cattle in Japan because of optimum mineral nutrition.

Furthermore, Zn, Cu, and Se concentrations in the liver of newborn calves born from cows fed low diet of those were higher than for cows, but Fe, Mn, Mo, and Co concentration of newborn calves born from cows fed adequate diet of those were lower. These data may be partly due to the effect of mineral interactions rather than the effect of mineral concentrations in maternal tissues, since the knowledge of the intimate relationships between or among minerals is just beginning to emerge in the fetus ( 63 ). However, it is not apparent whether trace element concentration in the liver, except for corresponding elements by each administration, was affected by trace element administration, although trace element metabolism in the body may be differed in each element. Therefore, further study may be needed on interrelationships that occur between or among minerals, since metabolic interrelationships between or among minerals have proved to be important in providing optimum nutrition for ruminants.

Table 40. Trace elements concentration in feed obtained from Kyushu Natl. Agric. Exp. Stn.  
and 10 dairy farms in Kumamoto prefecture

Feed	Number of samples	Fe <sup>1)</sup>	Zn <sup>1)</sup>	Cu <sup>1)</sup>	Mn <sup>1)</sup>	Se <sup>1)</sup>	Mo <sup>1)</sup>	Co <sup>1)</sup>	Cd <sup>1)</sup>
Concentrate	11	236 <u>±</u> 175 <sup>2)</sup>	56 <u>±</u> 6	10.1 <u>±</u> 2.3	36 <u>±</u> 13	0.19 <u>±</u> 0.05	0.79 <u>±</u> 0.18	0.10 <u>±</u> 0.06	0.22 <u>±</u> 0.04
		100-681 <sup>3)</sup>	46-68	7.0-14.5	20-67	0.13-0.32	0.50-1.03	0.05-0.22	0.15-0.29
Roughage	19	763 <u>±</u> 953	34 <u>±</u> 20	5.0 <u>±</u> 3.2	146 <u>±</u> 120	0.06 <u>±</u> 0.06	0.77 <u>±</u> 0.26	0.12 <u>±</u> 0.08	0.30 <u>±</u> 0.32
		69-3960	12-103	0.9-12.1	33-523	0.01-0.22	0.31-1.19	0.03-0.32	0.07-1.43
By-product feed	8	388 <u>±</u> 416	48 <u>±</u> 74	19.8 <u>±</u> 38.4	28 <u>±</u> 20	0.10 <u>±</u> 0.11	0.58 <u>±</u> 0.34	0.06 <u>±</u> 0.04	0.10 <u>±</u> 0.14
		19-1329	10-230	2.0-114.3	7-56	0.01-0.29	0.18-1.16	0.03-0.13	ND-0.38
Requirement ( NRC ) <sup>4)</sup>		50	40	10	40	0.10	---	0.10	---

1) ppm on dry matter basis. 2) Mean $\pm$ S.D. 3) Range. 4) Recommended nutrients contents of rations for dairy cattle. ND: below 0.01 ppm.

Table 41. Trace elements concentration in liver of Holstein cattle kept at Kyushu Natl. Agric. Exp. Stn. and 10 dairy farms in Kumamoto prefecture

Area	Kyushu Natl. Agric. Exp. Stn.				Dairy farm
	Newborn calf	Calf <sup>1)</sup>	Heifer <sup>2)</sup>	Cow <sup>3)</sup>	Cow <sup>4)</sup>
Number of samples	2	1	2	14	16
Fe <sup>5)</sup>	212 $\pm$ 259 <sup>6)</sup>	238	242 $\pm$ 219	781 $\pm$ 1023	260 $\pm$ 156
Zn <sup>5)</sup>	476 $\pm$ 141	541	254 $\pm$ 145	199 $\pm$ 73	156 $\pm$ 54
Cu <sup>5)</sup>	228 $\pm$ 5	192	92 $\pm$ 74	62 $\pm$ 56	137 $\pm$ 97
Mn <sup>5)</sup>	4.66 $\pm$ 1.17	9.73	10.32 $\pm$ 1.50	11.20 $\pm$ 1.65	7.98 $\pm$ 1.07
Se <sup>5)</sup>	1.50 $\pm$ 0.34	1.49	1.10 $\pm$ 0.01	1.00 $\pm$ 0.34	1.14 $\pm$ 0.14
Mo <sup>5)</sup>	3.34 $\pm$ 0.68	4.65	4.30 $\pm$ 0.08	4.63 $\pm$ 0.40	4.52 $\pm$ 0.40
Co <sup>5)</sup>	0.06 $\pm$ 0.01	0.06	0.16 $\pm$ 0.06	0.16 $\pm$ 0.07	0.16 $\pm$ 0.04
Cd <sup>5)</sup>	0.10 $\pm$ 0.06	0.08	0.22 $\pm$ 0.08	0.86 $\pm$ 0.38	0.71 $\pm$ 0.18

1) 1 month old. 2) 9 and 12 months old. 3) 29-144 months old.

4) 24-132 months old. 5) ppm on dry matter basis. 6) Mean $\pm$ S.D.

Table 42. Correlation coefficient of trace elements concentration in liver of Holstein cows kept at Kyushu Natl. Agric. Exp. Stn. and 10 dairy farms in Kumamoto prefecture

	Fe	Zn	Cu	Mn	Se	Mo	Co
Fe		-0.21 <sup>1)</sup>	-0.41	0.24	-0.69 <sup>**</sup>	-0.01	-0.31
Zn	0.21 <sup>2)</sup>		0.61 <sup>*</sup>	-0.01	0.37	0.06	0.17
Cu	0.35	0.65 <sup>**</sup>		0.03	0.80 <sup>**</sup>	-0.17	0.17
Mn	0.34	0.08	0.12		-0.06	-0.34	-0.42
Se	0.22	0.54 <sup>*</sup>	0.66 <sup>**</sup>	0.25		-0.32	0.23
Mo	-0.04	-0.23	0.24	0.32	0.30		-0.30
Co	0.39	-0.11	0.17	0.20	-0.17	0.13	

1) Kyushu Natl. Agric. Exp. Stn. ( n=14 ) 2) Dairy farm ( n=16 )

\*\* :  $P < 0.01$ , \* :  $P < 0.05$

Table 43. Trace element concentration in liver of trace element-treated cows

Treatment	Zn		Cu		Mn		Se		Mo		Co		Cd	
Cattle No.	7 <sup>1)</sup>	8 <sup>2)</sup>	9 <sup>1)</sup>	10 <sup>2)</sup>	11 <sup>1)</sup>	12 <sup>2)</sup>	13 <sup>1)</sup>	14 <sup>2)</sup>	15 <sup>1)</sup>	16 <sup>2)</sup>	17 <sup>1)</sup>	18 <sup>2)</sup>	19 <sup>1)</sup>	20 <sup>2)</sup>
Age ( mos. )	68	44	90	32	65	79	110	34	29	42	45	53	112	112
Body weight ( kg )	566	488	598	461	564	502	574	462	446	512	514	592	664	656
Fe	668 <sup>3)</sup>	379	454	291	230	542	549	331	471	342	367	510	471	240
Zn	207	159	122	114	167	154	139	119	122	119	143	131	185	135
Cu	10	31	368	273	131	78	23	51	30	94	118	134	76	60
Mn	10.1	10.7	8.9	10.2	10.3	18.5	12.2	14.1	11.6	13.3	9.5	11.3	10.5	11.2
Se	0.79	1.01	0.97	1.12	1.08	1.24	2.74	17.8	1.00	1.18	1.12	1.12	1.36	1.36
Mo	5.10	5.03	3.68	4.36	3.67	4.49	4.34	4.68	3.99	6.90	4.88	5.04	4.92	4.91
Co	0.16	0.06	0.23	0.16	0.14	0.08	0.19	0.14	0.15	0.18	0.15	0.55	0.10	0.11
Cd	0.53	0.54	0.76	0.52	0.82	0.53	0.78	0.38	0.40	0.41	0.59	0.46	13.8	15.3

1) slaughtered 4 weeks after the termination of the administration. 2) slaughtered immediately after the administration. 3) ppm on dry matter basis.

### Chapter 3. Effect of hot environmental temperature on trace element requirement of dairy cattle

#### Section 1. Effect of hot environmental temperature on trace element metabolism of dry cows during feeding and fasting

Heat stress in the summer environment in Japan causes significant declines in the feed intake, milk yield, and milk composition of dairy cattle ( 193, 194 ). Since dairy cattle increase their respiration rate and rectal temperature when exposed to hot temperature, it is important to prevent body temperature rise at high temperatures in order to maintain the same productivity as at optimal temperatures ( 16, 27, 193 ). Also, heat stress may increase K and Na requirement in dairy cattle, since the increase in K and Na loss via the skin, saliva, and urine under high temperatures has been reported ( 3, 27 ). Furthermore, there may be possible to increase the productivity of heat-stressed cattle when increased dietary Na and K, since increasing dietary Na and K may affect milk yield, milk composition, and the physiological and endocrine responses of cattle ( 27, 30, 31, 39, 115, 190, 191 ).

In the previous paper ( 110, 111 ), it is suggested that Ca, P, and Mg requirement for dry cows may increase in hot weather, since the maintenance requirement of those increases with temperatures above 27°C and/or apparent absorption decreases at 32°C. However, trace element requirements of cows in hot weather have not been well determined, although those in temperate conditions have been estimated for different classes using the factorial method ( 3, 149 ). Also, there may be the possibility of a borderline trace element deficiency for the cattle in Japan ( Chapter 2. ). Thus, an evaluation of trace element requirement in hot weather may be necessary to increase the productivity of dairy cows. This section was, therefore, initiated to evaluate the effect of hot environmental temperature on the

trace element metabolism of dry cows during feeding and fasting.

## Materials and Methods

Four dry, non-pregnant Holstein cows were used and housed in two independently controlled climatic rooms each housing two open-circuit respiration chambers ( 195 ). Each chamber was equipped with its own air-conditioning equipment in order to maintain a constant environment in the chamber. The controlled temperature and relative humidity ( RH ) of each chamber ranged from 10 to 40°C and 30 to 90%, respectively.

Trace element metabolism was determined under the following regime: 18, 27, and 36°C with 60% RH. Two of the cows were exposed to the temperatures in the order 18, 27, and 36°C, while the other two were exposed to the same temperatures in the reverse order. Before fasting began, all the cows were fed 7.2–8.2 kg Italian ryegrass hay at a level corresponding to their maintenance requirements in the chambers for 3 days, but their consumption was not measured. They were given their last at 17:00 h on the day preceding fasting measurement, which started at 13:00 h the following day and continued until 13:00 h on the 4th day. After measurement the cows were returned to the barn and fed the same hay for 10 days and then placed in the chamber again for the next treatment. Water was given ad libitum and measured during feeding and fasting. Live weights were measured at the beginning and end of fasting and also after 18 h and 66 h of fasting.

Heat production usually but not invariably decreased continuously until the 3rd day of fasting and slightly increased on the 4th day ( 195 ). Therefore, the fasting metabolism was taken to be the mean heat production per metabolic body size (  $\text{kg}^{.75}$  ) per day measured 68–116 h after food, a period referred to as the 3rd and 4th days of fasting although respiratory quotients declined continuously and reached 0.71 on the 4th

day ( 195 ). Thus, trace element metabolism of the cows during the fasting was measured 68–116 h after food, and feces and urine were collected over the same period. Feces and urine were also collected the day before fasting, and it assumed as trace element metabolism during the feeding although feeding period was quite short.

Samples of feces and hay were dried in a 60°C forced air oven and ground by stainless-steel Wiley mill. Urine samples were frozen before analysis. Samples were digested in nitric-perchloric acid, and Fe, Zn, Cu, and Mn were determined by atomic absorption spectrophotometry. Selenium was determined by fluoremetric analysis ( 234 ) and Mo and Co by graphite furnace atomic absorption spectrophotometry ( 119 ).

The data were analyzed by the library program of the Computing Centre for Research in Agriculture, Forestry and Fishery ( 161, 167 ).

## Results and Discussion

There were no significant differences in body weight between the treatments both during feeding and fasting ( Table 44 ). However, body weight at 36°C during fasting was highest, although during feeding it was lowest. Hay intake decreased at high temperatures during feeding, but water intake during feeding and fasting increased with temperature. Also, the degree of increase in water intake during fasting was greater than that during feeding, although water intake during fasting was significantly lower. It is reported that heat stress in cattle results in increased water intake and decreased roughage intake ( 27, 194 ). Furthermore, heat production during feeding increased by 18% at 27°C and 10% at 36°C compared with that for the 18°C treatment, and fasting metabolism tended to increase slightly at 36°C, but the differences between the treatments were not significant ( 195 ). Thus, the lowest body weight at 36°C during feeding



may be due to decreased hay intake and increased heat production, but the reason for the highest body weight at 36°C during fasting is not clear, although water intake did increase rapidly at 36°C.

Trace element concentrations in hay were as follows ( ppm on dry matter basis ): Fe, 481; Zn, 13; Cu, 2.0; Mn, 88; Se, 0.08; Mo, 0.97; Co, 0.07. Those in water were as follows ( ppm ): Fe, 0.01; Zn, 0.13; Cu, 0.006; Mn, 0.002; Se, 0.0001; Mo, <0.001; Co, <0.001. Zinc, Cu, Se, and Co concentrations in hay were lower than the NRC recommendations ( 149 ), although Fe and Mn concentrations were higher. Thus, Zn, Cu, Se, and Co intake of the cows was marginally inadequate.

There were no significant differences in Fe excretion between the treatments during feeding and fasting ( Table 45 ). Iron excretion tended to increase with temperature during fasting, but during feeding it decreased at 36°C. Iron intake from water increased with temperature during fasting, but compared to Fe excretion it was very low.

There were no significant differences in Zn excretion between the treatment during fasting, although urinary Zn excretion decreased at high temperatures ( Table 46 ). However, Cu excretion during fasting increased with temperature, especially at 27°C ( Table 47 ). Also, Zn and Cu excretion during feeding decreased at 36°C. According to ARC standards ( 3 ), the mean values of the endogenous Zn and Cu loss of cattle are 0.045 and 0.0071 mg/kg body weight per day respectively, but those of other trace elements were not estimated. Compared with Zn and Cu excretion during fasting, the endogenous Zn and Cu loss of ARC ( 3 ) are much lower. However, fecal Ca, P, and Mg excretions during fasting were slightly lower than the endogenous fecal loss of Ca, P, and Mg of ARC, and those might be regarded as the assumed value of the net minimum requirement for maintenance ( 110 ). The reason for the high value of Zn and Cu excretion during fasting is not clear, but further study may be necessary to evaluate

the relationships between the net minimum maintenance requirement of trace elements and their excretion during fasting.

Manganese excretion during fasting as well as Fe and Cu increased rapidly at 27°C, but was not significantly different ( Table 48 ). Also, Se, Mo, and Co excretion during fasting tended to increase with temperature, but their excretion during feeding decreased at 36°C ( Table 49-51 ). Since the upper critical temperature of dry cows is defined at 25°C ( 27 ), trace element excretion during fasting as well as major mineral excretion may increase with increasing temperature above 27°C, although Zn and Se excretion at 27°C were almost similar to that at 18°C. Thus, increased trace element excretion with temperature during fasting may be due to increased requirements, although decreased excretion at 36°C during feeding may be due to decreased intake. Also, feces may be a major pathway of trace element excretion during feeding and fasting in cattle, since fecal excretion of trace elements during feeding and fasting was significantly greater than urinary excretion.

It is suggested that heat-stressed dairy cows may have increased K and Na requirement ( 3, 27 ), and major mineral requirements, especially the maintenance requirement, may increase with temperature above 27°C ( 110 ). This experiment indicates that trace element requirements for maintenance of cows may be affected by heat stress and increase with temperature above 27°C. However, it is uncertain whether results from acute heat stress in an environmental chamber are wholly applicable to the chronic conditions in the actual field ( 27 ). Therefore, further study is needed to establish the trace element requirements of heat-stressed dairy cows.

Table 44. Body weight and water intake of cows during feeding and fasting  
on different environmental temperature

Item	Treatment means			Treatment effect	l.s.d. (P<0.05)
	18°C	27°C	36°C		
----- During feeding -----					
Body weight, kg	478.9	474.3	466.3	NS	
Water intake, kg/day	29.34	32.92	35.83	NS	
----- During fasting -----					
Body weight, kg	397.5	395.5	402.9	NS	
Water intake, kg/day	2.96	4.94	18.64	*	9.85

Level of significance: \*  $P < 0.05$ ; NS Not significant.

Table 45. Iron intake and excretion of cows during feeding and fasting on different environmental temperature

Item	Treatment means			Treatment effect	l.s.d. (P<0.05)
	18°C	27°C	36°C		
----- During feeding -----					
Excretion, mg/kg body wt per day					
Feces	9.73	12.05	8.52	NS	
Urine	0.071	0.063	0.067	NS	
Total	9.80	12.11	8.59	NS	
----- During fasting -----					
Intake through water, $\mu$ g/kg body wt per day	0.07	0.12	0.44	**	0.19
Excretion, mg/kg body wt per day					
Feces	3.31	4.10	3.94	NS	
Urine	0.018	0.029	0.028	NS	
Total	3.33	4.13	3.97	NS	

Level of significance: \*\* P<0.01; NS Not significant.

Table 46. Zinc intake and excretion of cows during feeding and fasting on different environmental temperature

Item	Treatment means			Treatment effect	l.s.d. (P<0.05)
	18°C	27°C	36°C		
----- During feeding -----					
Excretion, mg/kg body wt per day					
Feces	0.455	0.505	0.403	*	0.057
Urine	0.034	0.014	0.020	NS	
Total	0.489	0.520	0.422	*	0.049
----- During fasting -----					
Intake through water, µg/kg body wt per day	0.962	1.583	5.772	**	2.512
Excretion, mg/kg body wt per day					
Feces	0.160	0.165	0.172	NS	
Urine	0.009	0.004	0.006	*	0.003
Total	0.169	0.169	0.178	NS	

Level of significance: \*\* P<0.01; \* P<0.05; NS Not significant.

Table 47. Copper intake and excretion of cows during feeding and fasting on different environmental temperature

Item	Treatment means			Treatment effect	l.s.d. (P<0.05)
	18°C	27°C	36°C		
----- During feeding -----					
Excretion, mg/kg body wt per day					
Feces	0.061	0.078	0.055	*	0.014
Urine	0.0008	0.0015	0.0012	NS	
Total	0.062	0.079	0.056	*	0.014
----- During fasting -----					
Intake through water, $\mu$ g/kg body wt per day	0.044	0.073	0.266	**	0.116
Excretion, mg/kg body wt per day					
Feces	0.0122	0.0177	0.0168	*	0.0041
Urine	0.0007	0.0008	0.0018	**	0.0004
Total	0.0129	0.0185	0.0186	*	0.0041

Level of significance: \*\* P<0.01; \* P<0.05; NS Not significant.

Table 48. Manganese intake and excretion of cows during feeding and fasting on different environmental temperature

Item	Treatment means			Treatment effect	l.s.d. (P<0.05)
	18°C	27°C	36°C		
----- During feeding -----					
Excretion, mg/kg body wt per day					
Feces	1.47	1.65	1.29	NS	
Urine	0.013	0.011	0.011	NS	
Total	1.48	1.66	1.30	NS	
----- During fasting -----					
Intake through water, $\mu\text{g/kg}$ body wt per day	0.014	0.024	0.089	**	0.039
Excretion, mg/kg body wt per day					
Feces	0.426	0.600	0.515	NS	
Urine	0.002	0.002	0.002	NS	
Total	0.428	0.602	0.517	NS	

Level of significance: \*\* P<0.01; NS Not significant.

Table 49. Selenium intake and excretion of cows during feeding and fasting on different environmental temperature

Item	Treatment means			Treatment effect	l.s.d. (P<0.05)
	18°C	27°C	36°C		
----- During feeding -----					
Excretion, µg/kg body wt per day					
Feces	0.793	0.676	0.698	NS	
Urine	0.093	0.074	0.079	NS	
Total	0.886	0.750	0.777	NS	
----- During fasting -----					
Intake through water, µg/kg body wt per day	0.0007	0.0012	0.0044	**	0.0019
Excretion, µg/kg body wt per day					
Feces	0.230	0.235	0.291	NS	
Urine	0.053	0.058	0.067	NS	
Total	0.283	0.293	0.358	NS	

Level of significance: \*\* P<0.01; NS Not significant.



Table 50. Molybdenum excretion during feeding and fasting on different environmental temperature

Item	Treatment means			Treatment effect	l.s.d. (P<0.05)
	18°C	27°C	36°C		
----- During feeding -----					
Excretion, µg/kg body wt per day					
Feces	6.180	5.669	4.956	NS	
Urine	0.446	0.443	0.646	NS	
Total	6.626	6.102	5.602	NS	
----- During fasting -----					
Excretion, µg/kg body wt per day					
Feces	1.243	1.564	1.435	NS	
Urine	0.193	0.188	0.198	NS	
Total	1.436	1.752	1.633	NS	

Level of significance: NS Not significant.

Table 51. Cobalt excretion during feeding and fasting on different environmental temperature

Item	Treatment means			Treatment effect	l.s.d. (P<0.05)
	18°C	27°C	36°C		
----- During feeding -----					
Excretion, µg/kg body wt per day					
Feces	3.272	4.055	2.805	NS	
Urine	0.044	0.073	0.060	NS	
Total	3.317	4.128	2.868	NS	
----- During fasting -----					
Excretion, µg/kg body wt per day					
Feces	0.764	0.832	0.859	NS	
Urine	0.014	0.009	0.012	NS	
Total	0.778	0.841	0.872	NS	

Level of significance: NS Not significant.

## Section 2. Effect of hot environmental temperature on trace element balance of lactating cows

Heat stress reduces feed intake, milk yield, and milk composition of dairy cattle ( 27, 193 ). In the previous paper ( 110-112 ), it is suggested that dietary major mineral requirements for dry cows may increase in hot weather and major mineral absorption of lactating cows may decrease above 26°C. Also, trace element requirements for maintenance of cows may increase in hot weather, and there may be the possibility of a borderline trace element deficiency for the cattle in Japan ( Chapter 2. ).

However, trace element requirements of lactating cows have not been estimated in hot weather. Therefore, it may be important to assess the trace element requirements of lactating cows in hot temperature in order to improve the productivity of lactating cows in hot weather. Thus, this section was conducted to evaluate the effect of hot environmental temperature on the trace element balance in lactating cows.

### Materials and Methods

Four lactating Holstein cows were used and housed in two independently controlled climatic rooms each housing 2 open-circuit respiration chambers ( 195 ). Trace element balance was determined under the following regime; 18, 26, and 30°C with 60% RH. Two of the cows were exposed to temperatures in the order, 18, 26, and 30°C for 2 weeks per treatment, while the other two were exposed to the same temperatures in reverse order. All the cows were fed concentrate and Italian ryegrass hay at a level corresponding to their requirement in the chambers and their consumption was measured. Concentrate contained ( in kg per 100 kg ): barley, 38.2; linseed meal 20.5; corn, 10.9; rice bran, 10.2; wheat bran, 10.2; soybean meal, 5.5;

calcium carbonate, 2.18; NaCl, 1.73; dicalcium phosphate, 0.49; and ( in g per 100 kg ):  $\text{FeSO}_4$ , 8.19;  $\text{MnSO}_4$ , 7.92;  $\text{CuSO}_4$ , 0.82;  $\text{ZnSO}_4$ , 0.37;  $\text{CoCl}_2$ , 0.05; KI, 0.04. Immediately after having been measured for 2 weeks, they were placed again in the chambers for the next treatment. Water and block salt was given ad libitum and their intake was measured. Block salt contained the following minerals ( in g per 100 kg ):  $\text{CaCO}_3$ , 500; S, 400;  $\text{MgCO}_3$ , 184.5;  $\text{Fe}_2\text{O}_3$ , 143; ZnO, 12;  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 39;  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 36;  $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ , 48; KI, 20;  $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ , 24, and NaCl was residual. Body weight was measured every week during each treatment.

Feces and urine were separately collected daily during the 7-d sampling period of the 2nd week during each treatment. Milk yield was measured twice a day and milk samples were collected daily during the 4-d sampling period of the 2nd week during each treatment. Feed samples were taken at each treatment. The preparation of samples, except for milk, and analyses were conducted as previously described ( Section 1 ). Milk samples were freeze-dried, ground by mortar, and then determined.

The data were analysed via the library program of Computing Centre for Research in Agriculture, Forestry and Fisheries ( 167 ).

## Results and Discussion

Body weight and daily milk yield significantly decreased with increasing temperatures ( Table 52 ). Hay intake significantly decreased with higher temperature, and concentrate and water intake also decreased with higher temperature but not significantly different. The decrease rate of feed intake with increasing temperatures was almost similar to that of milk yield, although that of hay intake was slightly greater than that of concentrate intake. Also, compared to previous experiment ( 111 ), water intake of lactating cows was greatly higher than that of dry cows and

tended to decrease with higher temperature, although that of dry cows increased with higher temperature. Thus, decreased milk yield with increasing temperatures may be mainly due to decreased feed and/or water intake affected by heat stress.

Block salt intake of lactating cows, as well as dry cows ( 111 ), decreased at high temperatures, although the treatment effect was not significant. However, block salt intake of lactating cows was lower than that of dry cows, probably because of higher consumption from supplemented mineral in concentrate.

Trace element concentrations in concentrate and hay were as follows ( ppm on a dry matter basis): Fe, 181, 674; Zn, 54, 38; Cu, 13.0, 7.9; Mn, 108, 121; Se, 0.34, 0.06; Mo, 0.31, 0.64; Co, 0.26, 0.35, respectively. Those in water were as follows ( ppm ): Fe, 0.01; Zn, 0.2; Cu, 0.006; Mn, 0.002; Se, 0.0003; Mo, <0.001; Co, <0.001. Thus, dietary levels of those obtained from feed, block salt, and water were as follows ( ppm on a dry matter basis ): Fe, 334-351; Zn, 50-52; Cu, 11.4-11.6; Mn, 112-113; Se, 0.25-0.26; Mo, 0.41-0.42; Co, 0.35-0.39. Therefore, dietary levels of trace element were almost adequate for lactating cows with respect to trace element nutrition, since dietary levels of those were higher than those recommended by NRC ( 149 ).

There were no significant differences in trace element concentrations, except for Mo, in milk between treatments, although Mo concentration in milk significantly decreased at 30°C ( Table 53 ). Also, except for Fe, trace element concentrations in milk at 26°C were comparatively high, but the reason for high concentrations in milk at 26°C is not clear. Thus, it is not apparent whether trace element concentrations in milk may be affected by heat stress, although those may be slightly affected in hot weather.

Iron and Zn intake significantly decreased with higher temperature

( Table 54 and 55 ). Since Fe and Zn excretion also significantly decreased with higher temperature, the decrease in Fe and Zn excretion was correlated with the decrease in these mineral intake. Also, Fe absorption and retention significantly decreased with temperature, but there were no significant differences in the Zn absorption and retention between treatment. However, Zn retention decreased at 26°C and that at 26°C was negative, since Zn secretion in milk at 26°C was relatively high.

The intake and excretion of Cu and Mn tended to decrease with higher temperature ( Table 56 and 57 ). Since Cu and Mn secretion in milk was very little, there were no significant differences in absorption and retention of those between treatments.

The intake and excretion of Se, Mo, and Co tended to decrease with higher temperature ( Table 58, 59, and 60 ). However, Se and Mo retention at 26°C as well as Zn was negative, since the ratio of secretion in milk to intake of Se and Mo was comparatively high and also the ratio of urine excretion to intake of Se and Mo was extremely greater than that of other trace elements. Also, there were no significant differences in Co absorption and retention between treatments, since Co secretion in milk was very low.

In this experiment, body weight, milk yield, feed intake, water intake, and block salt intake may be affected by heat stress and decrease above 26°C. Also, major mineral absorption may decrease above 26°C and decreased major mineral absorption above 26°C may be due to decreased secretion in milk of those ( 112 ). In this study, it is not apparent that the Cu, Mn, and Co balance of lactating cows may be affected by heat stress, but the Fe balance may be affected by heat stress since Fe absorption and retention decreased with higher temperature.

Also, the Zn, Se, and Mo balance may be affected by heat stress, since those retention at 26°C was negative. The reason for negative retention of

Zn, Se, and Mo at 26°C is not clear but it may be partly due to relatively high secretion in milk at 26°C. However, further study is needed on Zn, Se, and Mo nutrition for lactating cows, since those retention at 30°C was positive. Furthermore, more attention must be paid to Zn, Se, and Mo nutrition for lactating cows in relation to milk production, since the ratio of secretion in milk to intake of those as well as major mineral was extremely greater than that of Fe, Cu, Mn, and Co.

Heat stress influences the adverse effect on the productivity in dairy cattle ( 27, 193 ), and a marginal deficiency of trace elements is also likely to reduce milk production, growth rate, resistance to disease or infection, and reproduction ( 3, 142, 149, 230 ). Since Zn, Se, and Mo retention of lactating cows was negative at 26°C, those balance may be affected by heat stress in this study. Thus, it may be necessary to pay attention to interrelationships between heat stress and trace element nutrition for dairy cattle, since the interactions between heat stress and inadequate nutrition of trace elements may accelerate the adverse effect on the productivity of dairy cattle. Further study is, therefore, needed to establish the trace element requirements for heat-stressed cattle in order to increase the productivity of dairy cows in hot weather.

Table 52. Body weight, milk yield, concentrate intake, hay intake, water intake, and block salt intake of lactating cows on different environmental temperature

Item	Treatment means			Treatment effect	l.s.d. (P<0.05)
	18°C	26°C	30°C		
Body weight, kg	561.1	542.5	527.8	**	15.4
Milk yield, kg/day	27.51	23.33	19.29	**	3.92
Concentrate intake, kg/day	13.63	11.65	9.55	NS	
Hay intake, kg/day	7.01	5.23	4.24	*	1.49
Water intake, kg/day	89.12	81.01	71.69	NS	
Block salt intake, g/day	12.71	16.00	7.05	NS	

Level of significance: \*\* P<0.01; \* P<0.05; NS Not significant.



Table 53. Trace element concentration in milk of lactating cows  
on different environmental temperature

Item	Treatment means			Treatment effect	l.s.d. (P<0.05)
	18°C	26°C	30°C		
Fe, ppm	0.39	0.53	0.72	NS	
Zn, ppm	4.05	4.74	3.23	NS	
Cu, ppm	0.068	0.082	0.071	NS	
Mn, ppm	0.049	0.054	0.052	NS	
Se, ppm	0.026	0.034	0.026	NS	
Mo, ppm	0.032	0.045	0.016	*	0.017
Co, ppm	0.001	0.001	0.001	NS	

Level of significance: \* P<0.05; NS Not significant.

Table 54. Iron intake, excretion, secretion in milk, absorption, and retention of lactating cows on different environmental temperature

Item	Treatment means			Treatment effect	l.s.d. (P<0.05)
	18°C	26°C	30°C		
Intake, mg/kg body wt per day	11.334	9.027	7.490	**	1.928
Excretion, mg/kg body wt per day					
Feces	7.571	6.764	5.371	*	1.240
Urine	0.085	0.083	0.068	NS	
Total	7.656	6.847	5.439	*	1.273
Secretion in milk, mg/kg body wt per day	0.019	0.024	0.027	NS	
Absorption, mg/kg body wt per day	3.763	2.263	2.119	*	1.346
Retention, mg/kg body wt per day	3.659	2.156	2.024	*	1.318

Level of significance: \*\* P<0.01; \* P<0.05; NS Not significant.

Table 55. Zinc intake, excretion, secretion in milk, absorption, and retention of lactating cows on different environmental temperature

Item	Treatment means			Treatment effect	l.s.d. ( $P < 0.05$ )
	18°C	26°C	30°C		
Intake, mg/kg body wt per day	1.605	1.380	1.169	*	0.289
Excretion, mg/kg body wt per day					
Feces	1.376	1.286	0.972	**	0.170
Urine	0.036	0.033	0.035	NS	
Total	1.412	1.319	1.006	**	0.174
Secretion in milk, mg/kg body wt per day	0.202	0.202	0.118	NS	
Absorption, mg/kg body wt per day	0.229	0.094	0.197	NS	
Retention, mg/kg body wt per day	-0.009	-0.141	0.044	NS	

Level of significance: \*\*  $P < 0.01$ ; \*  $P < 0.05$ ; NS Not significant.

Table 56. Copper intake, excretion, secretion in milk, absorption, and retention of lactating cows on different environmental temperature

Item	Treatment means			Treatment effect	l.s.d. (P<0.05)
	18°C	26°C	30°C		
Intake, mg/kg body wt per day	0.366	0.315	0.262	*	0.069
Excretion, mg/kg body wt per day					
Feces	0.337	0.288	0.226	*	0.062
Urine	0.006	0.007	0.006	NS	
Total	0.343	0.295	0.232	*	0.063
Secretion in milk, mg/kg body wt per day	0.0034	0.0035	0.0028	NS	
Absorption, mg/kg body wt per day	0.029	0.027	0.036	NS	
Retention, mg/kg body wt per day	0.020	0.017	0.027	NS	

Level of significance: \* P<0.05; NS Not significant.

Table 57. Manganese intake, excretion, secretion in milk, absorption, and retention of lactating cows on different environmental temperature

Item	Treatment means			Treatment effect	l.s.d. ( $P < 0.05$ )
	18°C	26°C	30°C		
Intake, mg/kg body wt per day	3.621	3.062	2.540	*	0.605
Excretion, mg/kg body wt per day					
Feces	3.007	2.374	1.951	NS	
Urine	0.024	0.016	0.012	NS	
Total	3.031	2.391	1.963	NS	
Secretion in milk, mg/kg body wt per day	0.0024	0.0023	0.0018	NS	
Absorption, mg/kg body wt per day	0.614	0.688	0.589	NS	
Retention, mg/kg body wt per day	0.588	0.670	0.575	NS	

Level of significance: \*  $P < 0.05$ ; NS Not significant.

Table 58. Selenium intake, excretion, secretion in milk, absorption, and retention of lactating cows on different environmental temperature

Item	Treatment means			Treatment effect	l.s.d. (P<0.05)
	18°C	26°C	30°C		
Intake, $\mu\text{g/kg}$ body wt per day	7.977	7.001	5.813	NS	
Excretion, $\mu\text{g/kg}$ body wt per day					
Feces	5.434	5.356	3.655	*	1.484
Urine	1.193	1.228	1.146	NS	
Total	6.627	6.584	4.801	NS	
Secretion in milk, $\mu\text{g/kg}$ body wt per day	1.280	1.328	0.987	NS	
Absorption, $\mu\text{g/kg}$ body wt per day	2.543	1.645	2.158	NS	
Retention, $\mu\text{g/kg}$ body wt per day	0.070	-0.911	0.025	NS	

Level of significance: \* P<0.05; NS Not significant.

Table 59. Molybdenum intake, excretion, secretion in milk, absorption, and retention of lactating cows on different environmental temperature

Item	Treatment means			Treatment effect	l.s.d. (P<0.05)
	18°C	26°C	30°C		
Intake, $\mu\text{g/kg}$ body wt per day	13.565	11.238	9.330	**	2.062
Excretion, $\mu\text{g/kg}$ body wt per day					
Feces	10.074	8.519	6.878	**	1.278
Urine	1.310	1.027	0.899	NS	
Total	11.384	9.545	7.778	**	1.567
Secretion in milk, $\mu\text{g/kg}$ body wt per day	1.467	1.872	0.596	**	0.636
Absorption, $\mu\text{g/kg}$ body wt per day	3.491	2.719	2.452	NS	
Retention, $\mu\text{g/kg}$ body wt per day	0.714	-0.180	0.957	NS	

Level of significance: \*\* P<0.01; NS Not significant.

Table 60. Cobalt intake, excretion, secretion in milk, absorption, and retention of lactating cows on different environmental temperature

Item	Treatment means			Treatment effect	l.s.d. (P<0.05)
	18°C	26°C	30°C		
Intake, $\mu\text{g/kg}$ body wt per day	11.538	10.696	8.000	*	2.269
Excretion, $\mu\text{g/kg}$ body wt per day					
Feces	10.322	7.377	5.516	*	2.994
Urine	0.092	0.104	0.092	NS	
Total	10.414	7.481	5.608	*	2.993
Secretion in milk, $\mu\text{g/kg}$ body wt per day	0.058	0.052	0.044	*	0.009
Absorption, $\mu\text{g/kg}$ body wt per day	1.216	3.319	2.484	NS	
Retention, $\mu\text{g/kg}$ body wt per day	1.066	3.163	2.348	NS	

Level of significance: \* P<0.05; NS Not significant.



#### Chapter 4. Summary and Conclusion

Trace elements act primarily as a critical role of components in the body and catalysts in enzyme systems in the cells, and 16 trace elements are recognized as essential or probably essential for animal at the present. However, the requirement and tolerance of trace elements for dairy cattle has not been determined exactly, because there is little information in trace element nutrition of ruminant in Japan. Recently, it has been found that Zn, Cu, Se, and Co concentrations in most of Japanese forage were below the dietary requirement for dairy cattle. Therefore, it is necessary to clarify the trace element nutrition of dairy cattle in Japan, since there may be the possibility of trace element deficiency for dairy cattle in practical feeding in dairy farms.

Heat stress in summer environment in Japan causes significant declines in feed intake, milk yield, and milk composition of dairy cattle. Although heat-stressed cattle may have increased K and Na requirements, trace element requirements of cows in hot weather have not been well determined in Japan. Thus, an evaluation of trace element requirement of dairy cows in hot weather is necessary to increase the productivity of dairy cattle in Japan.

The objective of this study was, therefore, to clarify the effect of dietary trace elements level and hot environmental temperature on trace element nutrition of Holstein cattle. Firstly, this study was conducted to clarify the effect of dietary level of 8 trace elements on those status of 37 Holstein cattle obtained from Kyushu Natl. Agric. Exp. Stn. and 10 dairy farms in Kumamoto prefecture. Secondly, the ingestion, accumulation, distribution, and excretion of 7 trace elements in lactating cows was investigated by the administration of each element for lactating cows. At last, dry and lactating cows were exposed to hot temperature in order to

clarify the effect of hot environmental temperature on trace element metabolism of cows.

1. Effect of dietary trace element level on trace element nutrition of Holstein cattle

1) Iron intake by the cattle was almost adequate, since Fe concentration in feed was above the 50 ppm dietary requirement. Iron concentration in roughage, especially in silage, was sometimes above the 1000 ppm dietary tolerance.

2) There was appreciable storage of Fe in the spleen, lung, liver, and kidney. Also, most of dietary Fe seems to be excreted in the feces, since Fe concentration in the feces was higher than dietary Fe level.

3) Zinc intake by the cattle fed mainly roughage was marginally inadequate, since most Zn concentration in roughage was a little below the 40 ppm dietary requirement. Zinc concentration in the liver, kidney, and blood of the cows was not affected by dietary Zn level, but there may be the possibility of a borderline Zn deficiency for the cattle fed low Zn diet. Since Zn concentration in the feces increased with the increase in dietary Zn, most dietary Zn seems to be excreted in the feces.

4) Feed intake and daily milk yields of treated cows were not affected by the administration of 400 ppm Zn in the diet. Zinc concentration in the tissues of treated cows was almost similar to that for untreated cattle and most of Zn administered was excreted in the feces.

5) Copper intake by the cattle fed mainly roughage was marginally inadequate, since Cu concentration in roughage was almost below the 10 ppm dietary requirement. It seems likely that Cu deficiency may occur after long periods of feeding low Cu diet, since Cu concentration in the liver and blood decreased with reduced dietary Cu and liver Cu concentration in 5 cows which was below 10 ppm was regarded as Cu deficiency. Most dietary

Cu seems to be excreted in the feces, since Cu concentration in the feces increased with the increase in dietary Cu.

6) Feed intake and daily milk yields of treated cows were not affected by the administration of 75 ppm Cu in the diet, except for decreased appetite at the start of Cu administration. Liver Cu may be maintained for long periods comparatively, since Cu concentration in the liver of Cu-treated cows was very high and that of the cow slaughtered 4 weeks after the termination of the administration was higher than for untreated cattle. Most of Cu administered was excreted in the feces and slightly secreted in the milk.

7) Manganese intake by the cattle was sufficient, since dietary Mn level was almost above the 40 ppm dietary requirement. Manganese concentration in the liver, kidney, and blood was not affected by dietary Mn level. Most of dietary Mn seems to be excreted in the feces, since Mn concentration in the feces increased with the increase in dietary Mn.

8) Feed intake and daily milk yields of treated cows were not affected by the administration of 750 ppm Mn in the diet, except for slight decrease in feed intake at the start of Mn administration. Since Mn concentration in the tissues, milk, blood, and hair of Mn-treated cows was higher than for untreated cattle, some Mn may remain in the body. However, most of Mn administered was excreted in the feces.

9) Selenium intake by the cattle fed mainly roughage was marginally inadequate, since Se concentration in roughage was almost below the 0.1 ppm dietary requirement. It is suggested that Se deficiency in the cattle may occur after long periods of feeding low Se diet, since Se concentration in the liver and blood decreased with reduced dietary Se. Most dietary Se seems to be excreted in the feces, since Se concentration in the feces increased with the increase in dietary Se.

10) There was a decrease in feed intake, rapid salivation, and a swelling

of the cervix and sore feet of one treated cow by the administration of 6.5 ppm Se in the diet. It is suggested that most of Se administered was excreted in the feces and urine, and some Se was secreted in the milk. Also, some Se may remain for long periods in the body, since Se concentration in the liver, blood, and hair of the cow slaughtered 4 weeks after the termination of the administration was greater than for untreated cattle.

11) Molybdenum intake by the cattle was adequate, since Mo concentration in feed was below 1.2 ppm. Molybdenum concentration in the liver, kidney, blood, and feces of the cows was not affected by dietary Mo level.

12) Feed intake and daily milk yields of treated cows were not affected by the administration of 6.1 ppm Mo in the diet. It is suggested that most of Mo administered was excreted in the feces and urine, and slightly secreted in the milk, but some Mo accumulated in the body since higher Mo concentration of Mo-treated cows was obtained from most tissues.

13) Cobalt intake by the cattle was almost adequate, although Co concentration in feed was sometimes below the 0.1 ppm dietary requirement. However, there may be the possibility of a borderline Co deficiency for the cattle fed rations of low Co content. Cobalt concentration in the liver, kidney, blood, and feces was not affected by dietary Co level.

14) Feed intake and daily milk yields of treated cows were not affected by the administration of 3.4 ppm Co in the diet. It is suggested that most of Co administered was excreted in the feces and urine, but some Co accumulated in the body since higher Co concentration of Co-treated cows was obtained from the rumen, reticulum, omasum, liver, and kidney.

15) Cadmium intake by the cattle was almost adequate, since most feed contained less than 1 ppm Cd. Cadmium concentration in the liver and kidney increased with aging, but not in the other tissues.

16) Feed intake and daily milk yields of treated cows were not affected by

the administration of 83 ppm Cd in the diet. Most of Cd administered was excreted in the feces, but retained Cd was particularly accumulated in the liver and kidney.

17) Zinc, Cu, Se, and Co intake by the cattle fed rations of low those content as roughage was marginally inadequate, because those content in roughage was below the dietary requirement. There may be the possibility of the simultaneous occurrence of Zn, Cu, and Se deficiency for the cattle fed mainly roughage, since the correlation among those elements in the liver was significantly positive.

18) Zinc, Cu, and Se concentrations in the liver of newborn calves born from cows fed low those diet were higher than for cows, but Fe, Mn, Mo, and Co concentrations of newborn calves born from cows fed adequate those diet were lower. It is not apparent that trace element concentration in the liver, except for corresponding elements by each administration, was affected by the administration of each element.

## 2. Effect of hot environmental temperature on trace element requirement of dairy cattle

1) Hay intake of 4 dry cows decreased at high temperatures during feeding, but water intake during feeding and fasting increased with temperature.

2) Trace element excretion of dry cows during fasting was affected by heat stress and tended to increase with temperature, although their excretion during feeding decreased at 36°C. Feces may be major pathway of trace element excretion of dry cows during feeding and fasting. Trace element requirements for maintenance of cattle may be affected by heat stress and increase with increasing temperatures above 27°C.

3) Body weight, milk yield, and hay intake of 4 lactating cows significantly decreased with increasing temperature, and concentrate intake, water intake, and block salt intake tended to decrease at high temperatures. There were no significant differences in trace element

concentrations in milk between treatment, but Mo concentration in milk decreased at 30°C.

4) Iron absorption and retention of lactating cows decreased with higher temperatures, but there were no significant differences in the absorption and retention of Cu, Mn, and Co between treatment. Negative retention of Zn, Se, and Mo at 26°C in lactating cows may be partly due to relatively high secretion in milk at 26°C. The ratio of secretion in milk to intake of Zn, Se, and Mo in lactating cows was extremely greater than that of Fe, Cu, Mn, and Co. Iron, Zn, Se, and Mo balance in lactating cows may be affected by heat stress.

From the results obtained in this study, the following conclusion can be derived:

Trace element concentration in feed varied widely among different type or same type of feed. Also, Zn, Cu, and Se concentration in roughage was almost below the dietary requirement, although Fe and Mn concentration in feed was almost above the dietary requirement. Furthermore, Co concentration in feed was sometimes below the dietary requirement, and Mo and Cd concentration in feed was below the dietary tolerance. Therefore, Zn, Cu, Se, and Co intake by the cattle fed rations of low those content as roughage in Japan was marginally inadequate. Since variable feed was used in Japanese farms, it is necessary to pay attention to trace element nutrition in practical feeding of dairy cattle. Thus, sufficient trace elements should be added up to the dietary requirement in the total ration if low those diet were used. However, except in the case of environmental contamination in soil and feed, trace element concentration in Japanese feed may be almost below the dietary tolerable level for dairy cattle.

There may be the possibility of the simultaneous occurrence of Zn, Cu, and Se deficiency for the cattle fed low those diet as roughage, since

the correlation among those elements in the liver was significantly positive and those concentration in the liver decreased with reduced dietary level of those. Also, there may be mineral interactions between dam-fetus metabolism, since Zn, Cu, and Se concentration in the liver of newborn calves born from cows fed low those diet were higher than for cows and Fe, Mn, Mo, and Co concentration in the liver of newborn calves born from cows fed adequate those diets were lower. However, it is not apparent whether there is metabolic interaction among trace elements by trace element administration, although trace element accumulation, distribution, and excretion of lactating cows may be differed in each element. Therefore, more attention must be paid to mineral interaction as well as mineral metabolism in the body in feeding dairy cattle in Japan, since metabolic interrelationships between or among minerals have proved to be important in providing optimum mineral nutrition for dairy cattle.

Trace element requirements for maintenance of dairy cows may be affected by heat stress and increase above 27°C. Also, heat stress may influence Zn, Se, and Mo balance in lactating cows, and those nutrition of lactating cows may be more important in relation to milk production in hot weather. Thus, the interactions between heat stress and inadequate nutrition of trace elements in dairy cattle may accelerate the adverse effect on the productivity of dairy cattle. Therefore, it is needed on optimum supplementation of trace elements for dairy cows in summer environment in order to increase the productivity of lactating cows.

All plants and animals depend ultimately on the soil for their supply of mineral nutrients. Also, most dietary trace elements in dairy cattle were excreted in the feces and urine, since those concentration in the feces increased with the increase in dietary those level and most of trace elements administered was excreted in the feces and urine. It is, therefore, suggested that the soil-plant-animal interrelations of trace

elements should be taken into account carefully in optimum supplement of trace elements for dairy cattle, since a borderline deficiency or toxicity appeared possible where animals were fed rations in which trace element was deficient or concentrated to fertilize the soil as compost. However, trace element concentration in the milk and muscle may be kept in low level with and without the treatment of trace element administration, since a little trace element was moved in the milk and muscle by the administration.



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## ホルスタイン種乳牛の微量元素栄養におよぼす給与飼料中の 微量元素含量及び高温環境の影響

要約 微量元素は、主に体成分として、あるいは細胞内の酵素系における触媒として、動物体内で重要な役割をはたしているが、現在、動物に必須、あるいは多分必須と考えられている微量元素は16元素とされている。しかし、我が国では反芻動物の微量元素栄養に関する情報が不足しているため、乳牛の微量元素要求量や許容量はまだ明確に定められていないのが現状である。また、近年の調査によると、我が国の大部分の牧草のZn、Cu、Se及びCo含量が、乳牛の要求量以下であることが明らかにされている。そのため、実際の酪農家の乳牛飼養においても乳牛に微量元素が不足している可能性が強いので、我が国でも乳牛の微量元素栄養の実態を明らかにすることが必要とされている。

一方、我が国の夏期の暑熱ストレスは、乳牛の飼料摂取量、乳量及び乳成分の低下をもたらす。また、暑熱ストレスを被った牛では飼料中のK及びNa要求量の増加することが知られているが、高温環境下の乳牛の微量元素要求量については我が国ではまだほとんど研究されていない。そのため、我が国の乳牛の生産性を向上させるためには、高温環境下の乳牛の微量元素要求量を求めることが必要である。

本研究は、それゆえ、ホルスタイン種乳牛の微量元素栄養におよぼす飼料中の微量元素含量及び高温環境の影響を明らかにするためになされたものである。初めに、九州農業試験場及び熊本県内の10戸の酪農家で飼養されていた、37頭の乳牛の微量元素栄養におよぼす飼料中の微量元素含量の影響を明らかにした。次に、泌乳牛に微量元素を経口投与することによって、乳牛の微量元素の摂取、蓄積、分布及び排泄の様相について調査した。最後に、乳牛の微量元素代謝におよぼす高温環境の影響を明らかにするために、乾乳牛及び泌乳牛を高温に暴露させる実験を行った。

### 1.ホルスタイン種牛の微量元素栄養におよぼす飼料中の微量元素含量の影響

1)飼料のFe濃度は乳牛のFe要求量の50ppmを超えていたので、乳牛のFe摂取量はほぼ充足していた。粗飼料、特にサイレーズのFe濃度は、乳牛のFe許容量の1000ppmを超える値もみられた。

2)脾臓、肺臓、肝臓及び腎臓のFe濃度は、他の組織よりも高い値を示した。糞中のFe濃度が飼料中のFe含量よりも高い値を示したので、摂取したFeの大部分は糞中に排泄されるものと思われた。

3)大部分の粗飼料のZn濃度は乳牛のZn要求量の40ppmよりも低い値を示したので、粗飼料主体で飼養している牛のZn摂取量はやや不足していた。肝臓、腎臓及び血液のZn濃度は飼料中のZn含量による影響は認められなかったが、低Zn含量の飼料で飼養した牛はZn欠乏症の発生する可能性のあることが推察される。糞中のZn濃度は飼料中のZn含量の増加とともに増加したことから、摂取したZnの大部分は糞中に排泄されるものと思われ

た。

4)飼料中のZn含量が400ppmとなるようにZnを泌乳牛に投与しても、投与牛の飼料摂取量及び乳量にはZn投与による影響は認められなかった。投与牛の各組織のZn濃度は非投与牛のそれとほぼ同じ値を示し、また投与したZnの大部分は糞中に排泄された。

5)大部分の粗飼料のCu濃度は乳牛のCu要求量の10ppm よりも低い値を示したので、粗飼料主体で飼養した牛のCu摂取量はやや不足していた。肝臓及び血液のCu濃度は飼料中のCu含量の減少とともに減少し、また肝臓のCu濃度が10ppm 以下を示した 5頭の牛はCu欠乏の状態であったとみなされたので、長期にわたって低Cu含量の飼料を給与した牛はCu欠乏症が発生しやすいことが推察される。糞中のCu濃度は飼料中のCu含量の増加とともに増加したので、摂取したCuの大部分は糞中に排泄されるものと考えられた。

6)Cu投与開始時にやや食欲が減退したことを除けば、飼料中のCu含量が75ppm となるようにCuを泌乳牛に投与しても、投与牛の飼料摂取量及び乳量にはCu投与による影響は認められなかった。Cu投与牛の肝臓のCu濃度が非常に高濃度で、また投与終了 4週後に屠殺した牛の肝臓のCu濃度が非投与牛よりも高い値を示したので、肝臓に蓄積したCuは長期にわたって保持されることが推察される。投与したCuの大部分は糞中に排泄され、牛乳中への移行は微量であった。

7)大部分の飼料のMn濃度は乳牛のMn要求量の40ppm を超えていたので、乳牛のMn摂取量はほぼ充足していた。肝臓、腎臓及び血液のMn濃度は飼料中のMn含量による影響は認められなかった。糞中のMn濃度は飼料中のMn含量の増加とともに増加したので、摂取したMnの大部分は糞中に排泄されるものと考えられた。

8)Mn投与開始時にやや飼料摂取量が減少したことを除けば、飼料中のMn濃度が750ppm となるようにMnを泌乳牛に投与しても、投与牛の飼料摂取量及び乳量にはMn投与による影響は認められなかった。投与したMnの大部分は糞中に排泄されたが、Mn投与牛の各組織、牛乳、血液及び被毛のMn濃度が非投与牛のそれよりも高い値を示したので、投与したMnの一部が体内に移行したことが認められた。

9)大部分の粗飼料のSe濃度は乳牛のSe要求量の0.1ppmよりも低い値を示したので、粗飼料主体で飼養した牛のSe摂取量はやや不足していた。肝臓及び血液のSe濃度が飼料中のSe含量の減少とともに減少したので、長期にわたって低Se含量の飼料を給与した牛ではSe欠乏症の発生するおそれのあることが推察される。糞中のSe濃度は飼料中のSe含量の増加とともに増加したので、摂取したSeの大部分は糞中に排泄されるものと考えられた。

10)飼料中のSe含量が6.5ppmとなるようにSeを泌乳牛に投与することによって、1頭の投与牛には飼料摂取量の減少、急激な唾液分泌及び頸部と脚部の腫脹が発生した。投与したSeの大部分は糞尿中に排泄されたが、牛乳中への移行もみられた。また、投与終了 4週後に屠殺した牛の肝臓、血液及び被毛のSe濃度が非投与牛のそれよりも高い値を示したので、一部のSeは長期にわたって体内に保持されることが推察される。

11)飼料のMo濃度は1.2ppm以下であったので、乳牛のMo摂取量は適正なものと思われた。肝臓、腎臓、血液及び糞のMo濃度には、飼料中のMo含量の影響は認められなかった。

12)飼料中のMo含量が6.1ppmとなるようにMoを泌乳牛に投与しても、投与牛の飼料摂取量及び乳量にはMo投与による影響は認められなかった。投与したMoの大部分は糞尿中に排泄されたが、牛乳中への移行もみられた。また、投与牛の大部分の組織のMo濃度が非投与牛よりも高い値を示したことから、一部のMoが体内に移行したことが推察される。

13)一部の飼料のCo濃度に乳牛のCo要求量の0.1ppmよりも低い値がみられたが、乳牛のCo摂取量はほぼ充足していた。しかし、低Co含量の飼料で飼養した牛は、Co欠乏症の発生する可能性のあることが推察される。肝臓、腎臓、血液及び糞のCo濃度には、飼料中のCo含量による影響は認められなかった。

14)飼料中のCo含量が3.4ppmとなるようにCoを泌乳牛に投与しても、投与牛の飼料摂取量及び乳量にはCo投与による影響は認められなかった。投与したCoの大部分は糞尿中に排泄されたが、投与牛の第一胃、第二胃、第三胃、肝臓及び腎臓のCo濃度が高い値を示したので、一部のCoが体内に移行したことが推察される。

15)大部分の飼料のCd濃度が1ppm以下であったので、乳牛のCd摂取量はほぼ適正なものと思われた。肝臓及び腎臓のCd濃度は加齢とともに顕著な増加を示したが、他の組織では認められなかった。

16)飼料中のCd濃度が83ppmとなるようにCdを泌乳牛に投与しても、投与牛の飼料摂取量及び乳量にはCd投与による影響は認められなかった。投与したCdの大部分は糞中に排泄されたが、体内に移行したCdは肝臓及び腎臓への残留が顕著であった。

17)大部分の粗飼料のZn、Cu、Se及びCo濃度が乳牛の要求量よりも低い値を示したので、粗飼料主体で飼養した牛のそれらの摂取量はやや不足していた。肝臓のZn-Cu-Se間に有意な正の相関が得られたことから、粗飼料主体で飼養した牛ではZn、Cu及びSe欠乏症の同時に発生する可能性のあることが推察される。

18)Zn、CuおよびSe摂取量の少ない成牛から生まれた新生子牛の肝臓のZn、Cu及びSe濃度は、成牛のそれらよりも高い値を示したが、逆にFe、Mn、Mo及びCo摂取量のほぼ充足していた成牛から生まれた新生子牛の肝臓のFe、Mn、Mo及びCo濃度は、成牛のそれらよりも低い値を示した。投与牛の肝臓の微量元素濃度には、投与した元素の蓄積を除くと、微量元素投与による影響は認められなかった。

## 2.乳牛の微量元素要求量におよぼす高温環境の影響

1)採食時の4頭の乾乳牛の粗飼料摂取量は高温で減少したが、飲水量は採食時及び絶食時とも温度上昇とともに増加した。

2)乾乳牛の採食時の微量元素排泄量は36°Cで減少したが、絶食時のそれらは暑熱ストレスによって影響され、温度上昇とともに増加する傾向が示された。乾乳牛の採食時及び絶食時においては、糞中への排泄が微量元素の主要な排泄経路と思われる。乳牛の維持に要する微量元素要求量は暑熱ストレスによって影響され、27°Cを超えると温度上昇

とともに増加することが示唆される。

3) 4頭の泌乳牛の体重、乳量及び乾草摂取量は温度上昇とともに有意に減少し、また配合飼料摂取量、飲水量及び固形塩摂取量も温度上昇とともに減少する傾向がみられた。牛乳中のMo濃度は30°Cで減少したが、牛乳中の他の微量元素濃度には高温処理による影響は認められなかった。

4) 泌乳牛のFe吸収量及び蓄積量は高温で減少したが、Cu、Mn及びCo吸収量及び蓄積量は高温処理による影響は認められなかった。泌乳牛のZn、Se及びMo蓄積量が26°Cで負の値を示したことは、26°Cにおける牛乳中へのそれらの移行量が比較的多いことによるものと思われた。また、泌乳牛のZn、Se及びMoの摂取量に対する牛乳中への移行量の比率は、Fe、Cu、Mn及びCoのそれよりも非常に大きかった。泌乳牛のFe、Zn、Se及びMo出納は、暑熱ストレスによって影響されることが推察される。

以上の研究成果から、次の結論が導かれる：

飼料の微量元素濃度は、各飼料間、あるいは同一飼料内でも変動が非常に大きい。また、飼料のFe及びMn濃度は大部分が要求量を超えているが、粗飼料のZn、Cu及びSe濃度は大部分が要求量以下の値を示した。さらに、飼料のCo濃度は要求量以下の値もみられたが、Mo及びCd濃度は許容量よりも低い値を示した。それゆえ、Zn、Cu、Se及びCo濃度の低い粗飼料主体で飼養した牛は、それらの摂取量がやや不足の状態にある。また、我が国の酪農家は多種類の飼料を利用しているので、実際の乳牛飼養においては乳牛の微量元素栄養にも十分に注意することが必要である。さらに、微量元素含量の少ない飼料を給与していたならば、微量元素を補給することによって要求量を満たすことが必要である。しかし、我が国の飼料の微量元素濃度には、土壌や飼料への環境汚染によるケースを除けば、乳牛の許容量を超えるものはほとんどないと思われた。

肝臓のZn、Cu及びSe濃度間に正の有意な相関が得られ、また肝臓及び血液のCu及びSe濃度が飼料中のそれらの含量の減少とともに減少したので、Zn、Cu及びSe濃度の低い粗飼料主体で飼養した牛はそれらの欠乏症が同時に発生する可能性がある。また、Zn、Cu及びSe摂取量の少ない成牛から生まれた新生子牛の肝臓のZn、Cu及びSe濃度が成牛よりも高く、逆にFe、Mn、Mo及びCo摂取量の充足した牛から生まれた子牛では肝臓のFe、Mn、Mo及びCo濃度が成牛よりも低かったので、母親-胎児間の微量元素代謝には元素間に相互作用が働いていると思われる。しかし、体内における微量元素の蓄積、分布及び排泄の様相は各元素によって異なっていることが認められたが、微量元素を泌乳牛に投与することによっては、元素間の相互作用の働きを明らかにすることはできなかった。乳牛の微量元素栄養を適正に保つには、微量元素間の相互作用が微量元素代謝に重要な役割をはたすので、我が国でも実際の乳牛飼養においては体内における各元素の代謝と同様に各元素間の相互作用も十分に考慮することが必要である。

乳牛の維持に要する微量元素要求量は、暑熱ストレスによって影響され、高温環境下

では増加することが示唆される。また、暑熱ストレスは泌乳牛のZn、Se及びCu出納にも影響し、高温環境下の泌乳牛のそれらの栄養は、乳生産に関しては他の元素よりも重要なことが推察される。従って、乳牛の不適切な微量元素の栄養状態に暑熱ストレスが加わると、乳牛の生産性におよぼす悪影響をさらに加速することになる。そのため、泌乳牛の生産性を向上させるためには、夏期には乳牛に微量元素を補給することが必要である。

植物と動物は、ミネラル源の供給を最終的には土壤に依存している。また、飼料中の微量元素含量の増加とともに糞中の微量元素濃度が増加し、さらに投与した微量元素の大部分が糞尿中に排泄されたので、乳牛の飼料中の微量元素の大部分は糞尿中に排泄されるものと思われる。それゆえ、土壤に糞尿を堆肥として施肥することによって、飼料中の微量元素濃度の不足や濃縮が起こり、そしてその飼料を摂取した乳牛には欠乏症や中毒症の発生する可能性があるので、乳牛飼養においては土壤－植物－動物間の微量元素の相互関係についても十分に考慮しなければならない。しかし、微量元素投与による微量元素の牛乳や筋肉中への移行は非常に少ないので、牛乳及び筋肉の微量元素濃度は微量元素投与の有無にかかわらず低濃度に保たれると思われる。